

PGPR Amelioration in Sustainable Agriculture

Food Security and Environmental Management



Edited by
**Amit Kishore Singh, Ajay Kumar
and Pawan Kumar Singh**

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Edited by

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BIOGRAPHY

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Ecology and Diversity of Plant Growth Promoting Rhizobacteria in Agricultural Landscape

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1.1 INTRODUCTION

Currently the chemical fertilizers are used by farmers to supplement the essential nutrients to the soil associated plant system widely. The ease of availability and the environmental concerns of chemical fertilizers in special relation to the N fertilizers are real issues of today's agriculture. However, the use of chemical fertilizers has its own merits and demerits in agriculture land application and sustainable crop yields. Hence, there is an urgent requirement to alternative strategies in order to enhance the crop production and maintain the nutrient in the soil for ecological balance in agro-ecosystem. In the present scenario, the use of microbial inoculants or plant growth promoting rhizobacteria (PGPR) is promising and widely accepted practices in intensive agriculture for the sustainable agricultural production. PGPR are free-living soil bacteria that colonize root rhizosphere/of plant and promote the growth in terms of crop yields (Kumar et al., 2014a,b).

Earlier researcher has investigated that the rhizosphere is more diverse for bacteria than the surrounding bulk soil. These rhizospheric microbes derive benefit from the plant roots because it secretes metabolites that can be utilized as nutrients. It is reported that the bacterial population in the rhizosphere is found 10–1000 times higher than bulk soil (Lugtenberg and Kamilova, 2009). To exert their beneficial effects in the root environment, bacteria should have to be rhizosphere competent, that is, able to compete well with other rhizospheric microbes for nutrients secreted by

the roots. The discriminative use of food source by the microbes in root zone is not still well understood (Uren, 2007). An exception is the composition of the root exudates of tomato, in which organic acids, followed by sugars, are the major components (Lugtenberg et al., 2001). Study was carried out by the earlier researcher and they have confirmed the role of organic acids in root colonization and found that the mutants affected in organic acid utilization are poor competitive in root colonization compare to the parental strain (De Weert et al., 2007). It is reported that only a small part of the root surface is covered by bacteria, while, there is better chance of bacterial growth between epidermal cells and areas where side roots appear. This chapter highlights the diversity of PGPR and their potential exploitation in agricultural landscape in order to enhance the sustainable crop production.



1.2 MICROBIAL DIVERSITY ANALYSIS

Diversity of microorganisms is vital, as their unique characteristics can be utilized for crop improvement/production. Diversity of microorganism helps to build-up the ecosystem consists of microbe, soil, and plant. Functioning of this ecosystem is majorly governed by microbial dynamics (Kennedy and Smith, 1995). It is estimated that the microbial diversity in the soil is found to be in ranges of 10 billion of thousand different species. Discovering new microorganisms and characterizing their role in different areas are the major goal in the study of microbial diversity. Microorganism generally inhabited all parts of the plants from the root to the apical region of the plant, surface of the shoot (phyllosphere), and internal region of the plants (endophytes). In all forms most of these microbes help and promote the plant to live healthily and offer beneficial advantages to the plants. Among all these PGPR play an important role and are a central position in productivity and management of crop. Rhizospheric microbial diversity carries a variety of microorganisms which offer beneficial properties to the plant ecosystems. *Actinobacteria* a ubiquitous group of microorganism involved in decomposition of organic matter and suppression of soil borne plant pathogens (Altieri, 1991). The diversity analysis of the microorganisms present in soil enables us the information of predominant genera which could be further exploited in

agriculture by undergoing genetically manipulations. After genetic manipulation the predominant bacterial genera would play desired role in sustainable agriculture production.



1.3 PLANT GROWTH PROMOTING RHIZOBACTERIA

The plant growth promoting (PGP) effect of the PGPR is generally explained by the release of metabolites which directly promote the plant growth. There are several ways to explain the activities of PGPR benefit to the host plant. PGPR have potential to produce plant growth regulators such as indole acetic acid (IAA), cytokinins, and gibberellins (Glick, 1995; Marques et al., 2010), enhancing nitrogen fixation process (Sahin et al., 2004; Khan, 2005), promote solubilization of inorganic and organic phosphate (Bashan and de-Bashan, 2010). PGPR strains and their potential application in agricultural fields are summarized in Table 1.1.

On the basis of association of PGPR with the plant root cells, it is classified into two groups namely extracellular (ePGPR) and intracellular (iPGPR) (Martinez-Viveros et al., 2010). The ePGPR mostly found in the rhizosphere, rhizoplane, or spaces present between the cells of root cortex, conversely, iPGPR present generally inside the specialized nodular structures of root cells. Various bacterial genera such as *Agrobacterium*, *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Erwinia*, *Flavobacterium*, *Micrococcus*, *Pseudomonas* and *Serratia* belong to the ePGPR category (Gray and Smith, 2005; Bhattacharyya and Jha, 2012). The iPGPR shows activity with endophytes and *Frankia* species, which possess potential to fix atmospheric N₂ symbiotically with the higher plants (Jeon et al., 2003). PGPR also express to protect the plants against phytopathogenic microorganisms by the production of siderophores, synthesis of new antibiotics, enzymes, and/or also competes with detrimental microorganisms in the soil (Dey et al., 2004; Lucy et al., 2004). The PGPR which are associated with cereals, have increased attention due to their beneficial effects on growth and yield of different crops (Ozturk et al., 2003; Marques et al., 2010; Zhang et al., 2012). The inoculation with PGPR strain such as *Azotobacter* could help to reduce the use of nitrogen-based chemical fertilizer (Narula et al., 2005). More recently, Kumar et al. (2014a,b) conducted experiments on wheat under pot and field

Table 1.1 PGPR and their potential application in agriculture

Bacterial species	Host plant	Utilization	References
<i>Azospirillum</i> sp.	<i>Zea mays</i>	N ₂ fixation (rhizosphere)	Garcia de Salamone et al. (1996)
<i>Bacillus polymyxa</i>	<i>Triticum aestivum</i>	N ₂ fixation (rhizosphere)	Omar et al. (1996)
<i>Agrobacterium</i> sp.	<i>Lactuca sativa</i>	IAA production	Barazani and Friedman (1999)
<i>Pseudomonas fluorescens</i>	<i>Glycine max</i>	Cytokinin production	Garcia de Salamone et al. (2001)
<i>Bacillus</i> sp.	<i>Alnus</i>	Gibberelin production	Gutierrez-Manero et al. (2001)
<i>Herbaspirillum</i> sp.	<i>Oryza sativa</i>	N ₂ fixation (endophytic)	James et al. (2002)
<i>Azospirillum lipoferum</i>	<i>Triticum aestivum</i>	Promoting root development	Belimov et al. (2004)
<i>Herbaspirillum seropedicae</i>	<i>Oryza sativa</i>	Enhance production of gibberellins	Araujo et al. (2009)
<i>Azotobacter chroococcum</i>	<i>Triticum aestivum</i>	Phosphate solubilization	Bhattacharyya and Jha (2012)
<i>Azotobacter aceae</i>	<i>Fagopyrum esculentum</i>	N ₂ fixation	Bhattacharyya and Jha (2012)
<i>Azospirillum brasilense</i>	<i>Zea mays</i>	IAA production	Orlandini et al. (2014)
<i>Rhizobium leguminosarum</i>	<i>Phaseolus vulgaris</i>	Phosphate production	Ahemad and Kibret (2014)
<i>Bacillus subtilis</i>	<i>Hordeum vulgare</i>	Prevention of powdery mildews	Prathap and Ranjitha (2015)
<i>Paenibacillus polymyxa</i>	<i>Sesamum indicum</i>	Prevention of fungal disease	Ngumbi and Kloepper (2016)

condition to examine the effect of PGPR on the growth and yield of wheat and found that triple combination of strains *B. megaterium*, *A. chlorophenicus*, and *Enterobacter* significantly increased in plant height and yields of grain and straw. Majeed et al. (2015) reported effects of PGPR isolated from wheat rhizosphere. They observed that growth of wheat was promoted in the presence of PGPR.

The direct mechanism of PGPR is the major step involved to support plant growth in a forward and direct manner. Direct mechanism includes nitrogen fixation, phytohormones production, phosphate solubilization, and increasing iron availability. These mechanisms influence the plant growth activity directly but the ways by which it influences will vary from species to species as well as strain to strain. In the presence of PGPR

direct enhancement of mineral uptake has been reported due to increases in specific ion fluxes at the root surface (Bertrand et al., 2000). Organic substances that stimulate plant growth are known as plant growth regulators. They stimulate plant growth by influencing the physiological and morphological processes at very low concentrations (Arshad and Frankenberger, 1998). Several microorganisms are capable of producing auxins, cytokinins, gibberellins, ethylene (ET), or abscisic acid (ABA). Auxins are produced by several rhizobacterial genera, for example, *Azospirillum*, *Agrobacterium*, *Pseudomonas*, and *Erwinia* (Costacurta and Vanderleyden, 1995).



1.4 SPATIO-TEMPORAL CHANGES AND FACTOR AFFECTING PGPR DIVERSITY

Spatio-temporal variation may cause direct effects on the diversity of microbial community. Along with spatial separation, nutrient versatility also plays a key role in determining the microbial diversity in agricultural fields. There are various factors such as plants age, species, specific genotypes, and root exudates are the important key factors that directly affect the diversity of PGPR. In addition to this stresses, agricultural practices and ecological disturbances are the other exclusive factors that may affect the structure and diversity of soil microbial community. It is well proven by the earlier researchers that when soil is contaminated with heavy metal or other pollutants then there is marked decrease in both the biomass and diversity of the bacterial community occurs (Li et al., 2006; Yao et al., 2006; Castro-Sowinski et al., 2007). Such pressure of stress can potentially impact over the soil quality and crop productivity in negative manner. Other stresses such as desiccation, salinity, and temperature change affect microbial population structure (Castro-Sowinski et al., 2007).

Agricultural practices may also affect the soil microbiome but not impacted so much. However, it is well known that the agricultural practices cause alterations in soil parameters which may affect the rhizospheric microbial community (Castro-Sowinski et al., 2007). Castro-Sowinski et al. (2007) reported in various studies changes in microbial community and soil structure after tillage, crop rotation, and wastewater irrigation. However, after crop rotation increase in the bacterial diversity was observed in the rhizosphere by Lupwayi et al. (1998).



1.5 PHOSPHATE SOLUBILIZATION

Phosphorus is second most essential nutrient which is required by the plants in adequate amount for growth promotion. However, 95%–99% of phosphorus is present in insoluble, immobilized, or precipitated forms, hence, phosphorus is easily not available to the plants and unable to support plants. PGPR and fungi such as *mycorrhiza*, possess potential to solubilize and mineralize phosphorus and made easy for the plants (Richardson, 2001). It is reported that low molecular weight organic acids are generally synthesized by various soil bacteria and help in solubilization of inorganic phosphorus (Zaidi et al., 2009). On the contrary, the synthesis of a variety of different phosphorus made possible with the hydrolysis of phosphoric acid esters and leads to the mineralization process of organic phosphorus. Tao et al. (2008) investigated the bacteria that possess both phosphate solubilization and mineralization potential which could be useful for plant growth promotion. It is well established that most of the soils are poor in phosphorus content and farmers are also unable to use phosphate fertilizer in the field due its high cost. Hence, it is important to exploit the soil microorganisms as inoculum for phosphate mobilization in the field condition. Phosphate solubilizing bacteria such as *Bacillus*, *Rhizobium*, and *Pseudomonas* are the potent bacterial genera which are efficient to hydrolyze the inorganic phosphorus into soluble form and easily made available to the plant for growth promotion. Plants absorb phosphate in the form H_2PO_4^- and HPO_4^{2-} ions. Gouda et al. (2018) reviewed that solubilization and mineralization of phosphorus by phosphate-solubilizing bacteria is an important trait which could be achieved by the exploitation of PGPR strain. There is great variety of bacterial genera have been investigated as a phosphate solubilizing PGPR. These genera include *Arthrobacter*, *Bacillus*, *Burkholderia*, *Beijerinckia*, *Pseudomonas*, *Erwinia*, *Rhizobium*, *Mesorhizobium*, etc., have been used as soil inoculants by agriculture scientist enhance the plant growth and yield (Oteino et al., 2015; Kumar et al., 2014a,b, 2016). Among them, *Mesorhizobium ciceri* and *Mesorhizobium mediterraneum*, which are isolated from chickpea nodules, are good phosphate solubilizers (Parmar and Sindhu, 2013). Although these microbes solubilize phosphorus resulting in increased soil fertility, studies regarding their use as a bio-fertilizer are limited.

Among bacteria, the most efficient phosphate solubilizer belong to genera such as *Bacillus*, *Rhizobium*, and *Pseudomonas*. Within *Rhizobia*, two species nodulating chickpea, *Mesorhizobium ciceri* and *Mesorhizobium mediterraneum*, are known as good phosphate solubilizers (Rivas et al., 2006). In order to enhance the activity of the PGPR, it should be introduced into the soils. However, PGPR sometimes can be effective or sometimes completely inefficient due to the composition or variation in soils. Understanding of their mechanism and ecology in the rhizosphere could play a vital role in exploitation in the sustainable agriculture (Gyaneshwar et al., 2002). Goldstein (1995) reported that in order to lower down the pH of rhizospheric phosphate solubilizing bacteria have potential to dissolve the soil phosphate through production of low molecular weight organic acids such as gluconic and ketogluconic acids.



1.6 SIDEROPHORE PRODUCTION

Siderophores are small organic molecules which are generally formed by microorganisms under iron starvation conditions and then enhance their iron uptake potential. Various researchers have taken interest to carry out research in the last 10 years because of their unique characteristics to extract iron metal ions (Saha et al., 2016). Bacterial species such as *Pseudomonas sp.*, utilizes the siderophores during iron limiting condition, produced by other microbes which is present in the rhizosphere. Rathore (2015) reviewed that *Pseudomonas putida* possess potential to utilize heterologous siderophores that are produced by other microorganisms in the rhizosphere in order to enhance the level of iron available in the natural habitat (Rathore, 2015; Gouda et al., 2018). A potent siderophore, such as the ferric-siderophore complex, plays an important role in iron uptake by plants in the presence of other metals, such as nickel and cadmium (Beneduzi et al., 2012; Gouda et al., 2018). PGPR have potential to produce siderophores, which are important asset for providing the plant with the required amount of iron. However, more study yet to be explored regarding PGPR potential to produced siderophore.



1.7 NUTRIENT EXCHANGE

It is well established that plant's roots are the main source of carbon for the soil microorganisms. The carbon sources are derived either directly through root exudates, produced through photosynthesis, or via plant-residue inputs (Wolfe and Klironomos, 2005; Kaiser et al., 2015; Ishaq, 2017). Soil microorganisms possess inherent properties to contribute in nutrient cycling such as carbon, nitrogen, phosphorous, etc. via decomposition of biological detritus and other environmental elements in order to facilitate the efficacy of plant-microbs interactions (van der Heijden et al., 2016). It is well proven that compared to bulk soil, rhizosphere soil possess relatively more low-molecular-weight dissolved organic matter, 10 times more bacterial biomass, as well as higher diversity and abundance compare to bulk soil. (DeAngelis et al., 2008). Moreover, rhizosphere soil contains more bacterial quorum-sensing molecule *N*-acyl-homoserine lactone (AHL) (DeAngelis et al., 2008) which is mostly produced by the Proteobacteria phylum (Fray, 2002), suggesting that still more researches are required to explore larger concentrations of bacteria in the rhizosphere.



1.8 MICROORGANISMS IN AGRICULTURE

Before microorganisms were applied to agriculture, chemicals were used to enhance the crop yield and are still applied. These chemicals protect plant from pests and insects but were also able to enhance plant growth by overcoming the nutrition deficiency of the soil. Gradually, the adverse effect of these chemical is there in news for such a long time and has resulted in degradation of soil health. In cost, when microorganisms come into play, it carries many beneficial properties which impart to soil, plant growth, plant health, and environment. This uniqueness of microorganisms with biosynthetic potentials has made them the most suitable candidate to be applied to agriculture upon rigorous experimentation and validation of results. These microorganisms are effective when they provided optimum condition for growth and show beneficial effects on plants. Prime important activity of Agriculturally Important

Microorganisms (AIMs) is nitrogen fixation and make it available to plants, decomposition of organic matter in soil, detoxification of pesticides residues, protect plant from insects and pests or soil borne pathogens, enhance uptake of nutrients from soil, solubilization of nutrients unavailable to plants (e.g., N, P, K), produce hormone, vitamins, and enzymes, which enhance the growth of plants. AIMs have diverse applications in various areas of agriculture. AIMs are generally defined as a large group of frequently unknown or ill-defined microorganisms (Bhattacharyya et al., 2016) that interact favorably in soils with plants to render the beneficial effects which are sometimes difficult to predict. Microorganisms such as *Bradyrhizobium* sp., *Azotobacter chroococcum*, *Azospirillum basilensis*, *Paenibacillus* sp., *Pseudomonas* sp., *Rhizobium* sp., etc., have been established to show their potential in plant growth promotion. The microorganisms also play a key role in crop protection through the process of biocontrol which helps in disease resistance capacity of plants against phytopathogens, exhibiting antagonistic activities, or acting as biotic elicitors against different biotic and environmental factors (Singh et al., 2017a,b; Kumar et al., 2017a,b). The term “Effective Microorganism” is usually defined to a certain group of microbial cultures which is of beneficial in nature and is being used effectively as microbial inoculants that can be applied/aid in order to increase the diversity of native microbial community in soils and rhizospheric area of the growing plants. Microorganisms are valuable in managing the various types of insect’s pests, plant diseases, weeds, and other pests that usually damage the agricultural crops and forestry. Fungi are able to colonize in upper parts of the plants and provide benefits from drought as well heat tolerance, resistance to insects, and various plant diseases (Singh et al., 2011). The uniqueness of microorganisms, their unpredictable nature and biosynthetic capabilities make them quite adaptable in specific environment and cultural conditions to solve various problems related to crop improvement and disease suppression. Since microorganisms are useful in eliminating problems associated with the use of chemical fertilizers and pesticides, they have been widely applied in natural farming and organic agriculture (Russo et al., 2012). Microbial biotechnology and its application in the sustainable development of agriculture and environmental health are getting better attention. Growth promoting microorganisms protect plants under biotic or abiotic stress.



1.9 FUTURE PERSPECTIVE

Currently it is established that PGPR have potential to enhance the agricultural productivity via various routes and processes. However, there is a great deal of variation in the performance of PGPR that might be due to the environmental factors, which may affect their growth and exert their effects on plant. The environmental factors include climate change, soil characteristics, and the composite activity of the indigenous microbial flora of the soil (Gupta et al., 2015). Now, there are various modern tools and techniques such as biosensors, nanofertilizers in the fields of biotechnology, nanotechnology that have been applied in agriculture and allied sector for the enhancement in productivity and yields of crop. It is important to develop better insight of complex environment of the rhizosphere and associated bacteria, their mechanisms of action and exploitation of these PGPR in inoculant formulation, we could expect to see the feasibility and availability of the new PGPR strain in the rhizosphere. The success of getting new strain could enhance our ability to manage the rhizosphere and promote the survivality and competitiveness of these beneficial microorganisms. Potential strain could be managed via high throughput genetic engineering to improve the colonization efficacy and their effectiveness. The use of multistrain inoculums of PGPR with known functions is of the current interest as these formulations may increase consistency in the field. They offer the potential to address multiple modes of action, multiple pathogens, and temporal or spatial variability. PGPR offer an environmentally sustainable approach to increase crop production.

PGPR-based biotechnologies can be exploited for sustainable and eco-friendly technology for the management of plant stresses and biocontrol. It is reported that nano-based products and processes are being utilized by various developed countries such as United States, Germany, France, etc., for enhancing the agricultural growth and sustainable development. However, in India, there is urgent need to develop such useful technology to increase the agricultural food products which fulfilled to meet the requirements of large population.



1.10 CONCLUSIONS

The use of bacterial fertilizers has made significant improvement in terms of growth, health, and yield of plants. The mechanism by which

PGPR stimulates can be direct or indirect. PGPR also support growth by reducing the phytopathogens which reduce the yield and growth. The outcome of PGPR inoculation is greatly influenced by plant age and by the chemical, physical, and biological properties of the soil. There are several challenges for using PGPR such as natural variation but by the virtue of advance techniques and applying biotechnology can overcome the challenges. Hence future prospects can be replacement of chemical fertilizers and supporting the ecosystem in terms of safety. Further understanding of the complete mechanism of PGPR could help in obtaining more specific strain that will be able to work under more adverse and varying conditions.

Application of modern tools and techniques would be promising for the enhancement of PGPR strain that could play pivotal roles in sustainable agriculture by improving soil fertility, crop productivity, and management of nutrient cycle. Further, there is need to carry out more studies for selecting suitable rhizospheric microbial communities along with combination with interdisciplinary approaches in order to formulate their potential under field conditions.

REFERENCES

- Ahemad, M., Kibret, M., 2014. Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. *J King Saud Univ Sci* 26, 1–20.
- Altieri, M.A., 1991. How best can we use biodiversity in agroecosystems? *Outlook Agr* 20, 15–23.
- Araujo, A.S.F., Leite, L.F.C., Santos, V.B., Carneiro, R.E.V., 2009. Soil microbial activity in conventional and organic agricultural system. *Sustainability* 1, 268–276.
- Arshad, M., Frankenberger, W.T.J., 1998. Plant growth regulating substances in the rhizosphere: microbial production and functions. *Adv Agron* 62, 145–151.
- Barazani, O., Friedman, J., 1999. Is IAA the major root growth factor secreted from plant-growth-mediating bacteria? *J Chem Ecol* 25 (10), 2397–2406. Available from: <https://doi.org/10.1023/A:1020890311499>.
- Bashan, Y., de-Bashan, L.E., 2010. Chapter two—how the plant growth-promoting bacterium *Azospirillum* promotes plant growth—a critical assessment. *Adv Agron* 108, 77–136.
- Belimov, A.A., Kunakova, A.M., Safronova, V.I., Stepanok, V.V., Yu, L., Yu, Y., et al., 2004. Employment of rhizobacteria for the inoculation of barley plants cultivated in soil contaminated with lead and cadmium. *Microbiol* 73, 99–106.
- Beneduzi, A., Ambrosini, A., Passaglia, L.M.P., 2012. Plant growth-promoting rhizobacteria: their potential as antagonists and biocontrol agents. *Genet Mol Biol* 35 (4), 1044–1051.
- Bertrand, H., Plassard, C., Pinochet, X., Toraine, B., Normand, P., 2000. Stimulation of the ionic transport system in *Brassica napus* by a plant growth-promoting rhizobacterium. *Can J Microbiol* 46, 229–236.

- Bhattacharyya, P., Goswami, M., Bhattacharyya, L., 2016. Perspective of beneficial microbes in agriculture under changing climatic scenario: a review. *J Phytol* 8, 26–41.
- Bhattacharyya, P.N., Jha, D.K., 2012. Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. *World J Microbial Biotechnol* 28, 1327–1350.
- Castro-Sowinski, S., Herschkovitz, Y., Okon, Y., Jurkevitch, E., 2007. Effects of inoculation with plant growth-promoting rhizobacteria on resident rhizosphere microorganisms. *FEMS Microbiol Lett* 276, 1–11.
- Costacurta, A., Vanderleyden, J., 1995. Synthesis of phytohormones by plant associated bacteria. *Crit Rev Microbiol* 21, 1–18.
- De Weert, S., Kuiper, I., Kamilova, F., Mulders, I.H.M., Bloemberg, G.V., et al., 2007. The role of competitive root tip colonization in the biological control of tomato foot and root rot. In: Chincolcar, S.B., Mukerji, K.G. (Eds.), *Biological control of plant diseases*. Haworth, New York/London/Oxford, pp. 103–122.
- DeAngelis, K.M., Lindow, S.E., Firestone, M.K., 2008. Bacterial quorum sensing and nitrogen cycling in rhizosphere soil. *FEMS Microbiol Ecol* 66, 197–207.
- Dey, R., Pal, K.K., Bhatt, D.M., Chauhan, S.M., 2004. Growth promotion and yield enhancement of peanut (*Arachis hypogaea* L.) by application of plant growth promoting rhizobacteria. *Microbiol Res* 159, 371–394. Available from: <https://doi.org/10.1016/j.micres.2004.08.004>.
- Fray, R.G., 2002. Altering plant-microbe interaction through artificially manipulating bacterial quorum sensing. *Ann Bot* 89, 245–253.
- Garcia de Salamone, I.E., Dobereiner, J., Urquiaga, S., Boddey, R.M., 1996. Biological nitrogen fixation in *Azospirillum* strain-maize genotype associations as evaluated by the ¹⁵N isotope dilution technique. *Biol Fertil Soils* 23, 249–256. Available from: <https://doi.org/10.1007/BF00335952>.
- Garcia de Salamone, I.E., Hynes, R.K., Nelson, L.M., 2001. Cytokinin production by plant growth promoting rhizobacteria and selected mutants. *Can J Microbiol* 47, 404–411.
- Glick, B.R., 1995. The enhancement of plant growth by free-living bacteria. *Can J Microbiol* 4, 1109–1114.
- Goldstein, A.H., 1995. Recent progress in understanding the molecular genetics and biochemistry of calcium phosphate solubilization by Gram-negative bacteria. *Biol Agric Hort* 12, 185–193.
- Gouda, S., Kerry, R.G., Das, G., Paramithiotis, X., Shin, H.S., Patra, J.K., 2018. Revitalization of plant growth promoting rhizobacteria for sustainable development in agriculture. *Microbiol Res* 206, 131–140.
- Gray, E.J., Smith, D.L., 2005. Intracellular and extracellular PGPR: commonalities and distinctions in the plant-bacterium signaling processes. *Soil Biol Biochem* 37, 395–412.
- Gupta, G., Parihar, S.S., Ahirwar, N.K., Snehi, S.K., Singh, V., 2015. Plant growth promoting Rhizobacteria (PGPR): current and future prospects for development of sustainable agriculture. *J Microbiol Biochem* 7, 96–102.
- Gutierrez-Manero, F.J., Ramos-Solano, B., Probanza, A., Mehrouachi, J., Tadeo, F.R., Talon, M., 2001. The plant-growth promoting rhizobacteria *Bacillus pumilus* and *Bacillus licheniformis* produce high amounts of physiologically active gibberellins. *Physiol Plantarum* 111, 206–211.
- Gyaneshwar, P., Kumar, G.N., Parekh, L.J., Poole, P.S., 2002. Role of soil microorganisms in improving P nutrition of plants. *Develop Plant Soil Sci* 95, 133–143.
- Ishaq, S.L., 2017. Plant-microbial interactions in agriculture and the use of farming systems to improve diversity and productivity. *AIMS Microbiol* 3 (2), 335–353.

- James, E.K., Gyaneshwar, P., Mathan, N., Barraquio, W.L., Reddy, P.M., Iannetta, P.P.M., et al., 2002. Infection and colonization of rice seedlings by the plant growth-promoting bacterium *Herbaspirillum seropedicae* Z67. *Mol Plant Microbe Interact* 15, 894–906.
- Jeon, J.S., Lee, S.S., Kim, H.Y., Ahn, T.S., Song, H.G., 2003. Plant growth promotion in soil by some inoculated microorganisms. *J Microbiol* 41, 271–276.
- Kaiser, C., Kilburn, M.R., Clode, P.L., Fuchslueger, L., Koranda, M., Cliff, J.B., et al., 2015. Exploring the transfer of recent plant photosynthates to soil microbes: mycorrhizal pathway vs direct root exudation. *New Phytol* 205, 1537–1551.
- Kennedy, A.C., Smith, K., 1995. Soil microbial diversity and the sustainability of agricultural soils. In: H.P. Collins, G. P. Robertson, and M. J. Klug (Eds.), *The significance and regulation of soil biodiversity*. Kluwer Academic Publishers, pp. 75–86. Available from: https://doi.org/10.1007/978-94-011-0479-1_6.
- Khan, A.G., 2005. Role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation. *J Trace Elem Med Biol* 18, 355–364. Available from: <https://doi.org/10.1016/j.jtemb.2005.02.006>.
- Kumar, A., Maurya, B.R., Raghuwanshi, R., 2014a. Isolation and characterization of PGPR and their effect on growth, yield and nutrient content in wheat (*Triticum aestivum* L.). *Biocatal Agric Biotechnol* 3, 121–128.
- Kumar, A., Singh, R., Giri, D.D., Singh, P.K., Pandey, K.D., 2014b. Effect of *Azotobacter chroococcum* CL13 inoculation on growth and curcumin content of turmeric (*Curcuma longa* L.). *Int J Curr Microbiol Appl Sci* 3 (9), 275–283.
- Kumar, A., Singh, V., Singh, M., Singh, P.P., Singh, S.K., Singh, P.K., et al., 2016. Isolation of plant growth promoting rhizobacteria and their impact on growth and curcumin content in *Curcuma longa* L. *Biocatal Agr Biotechnol* 8, 1–7.
- Kumar, A., Verma, H., Singh, V.K., Singh, P.P., Singh, S.K., Ansari, W.A., et al., 2017a. Role of *Pseudomonas* sp. in sustainable agriculture and disease management. *Agriculturally Important Microbes for Sustainable Agriculture*. Springer, Singapore, pp. 195–215.
- Kumar, A., Singh, A.K., Kaushik, M.S., Mishra, S.K., Raj, P., Singh, P.K., et al., 2017b. Interaction of turmeric (*Curcuma longa* L.) with beneficial microbes: a review. *3 Biotech* 7 (6), 357.
- Li, Z., Xu, J., Tang, C., Wu, J., Muhammad, A., Wang, H., 2006. Application of 16S rDNA-PCR amplification and DGGE fingerprinting for detection of shift in microbial community diversity in Cu-, Zn-, and Cd-contaminated paddy soils. *Chemosphere*, 8, 1374–1380.
- Lucy, M., Reed, E., Glick, B.R., 2004. Application of free living plant-promoting rhizobacteria. *Antonie van Leeuwenhoek* 86, 1–25. Available from: <https://doi.org/10.1023/B:ANTO.0000024903.10757.6e>.
- Lugtenberg, B., Kamilova, F., 2009. Plant growth promoting rhizobacteria. *Annu Rev Microbiol* 63, 541–556.
- Lugtenberg, B.J.J., Dekkers, L.C., Bloembergen, G.V., 2001. Molecular determinants of rhizosphere colonization by *Pseudomonas*. *Annu Rev Phytopathol* 39, 461–490.
- Lupwayi, N.Z., Rice, W.A., Clayton, G.W., 1998. Soil microbial diversity and community structure under wheat as influenced by tillage and crop rotation. *Soil Biol Biochem*, 30, 1733–1741.
- Majeed, A., Abbasi, M.K., Hameed, S., Imran, A., Rahim, N., 2015. Isolation and characterization of plant growth-promoting rhizobacteria from wheat rhizosphere and their effect on plant growth promotion. *Front Microbiol* 6, 198. Available from: <https://doi.org/10.3389/fmicb.2015.00198>.
- Marques, A.P.G.C., Pires, C., Moreira, H., Rangel, A.O.S.S., Castro, P.M.L., 2010. Assessment of the plant growth promotion abilities of six bacterial isolates using *Zea*

- mays as indicator plant. *Soil Biol Biochem*, 42, 1229–1235. Available from: <https://doi.org/10.1016/j.soilbio.2010.04.014>.
- Martinez-Viveros, O., Jorquera, M.A., Crowley, D.E., Gajardo, G., Mora, M.L., 2010. Mechanisms and practical considerations involved in plant growth promotion by rhizobacteria. *J Soil Sci Plant Nutr* 10, 293–319.
- Narula, N., Kumar, V., Singh, B., Bhatia, R., Laxminarayana, K., 2005. Impact of biofertilizers on grain yield in spring wheat under varying fertility conditions and wheat-cotton rotation. *Arch Agron Soil Sci*. 51, 79–89. Available from: <https://doi.org/10.1080/03650340400029382>.
- Ngumbi, E., Kloepper, J., 2016. Bacterial-mediated drought tolerance: current and future prospects. *Appl Soil Ecol*, 105, 109–125.
- Omar, M.N.A., Mahrous, N.M., Hamouda, A.M., 1996. Evaluating the efficiency of inoculating some diazotrophs on yield and protein content of 3 wheat cultivars under graded levels of nitrogen fertilization. *Ann Agric Sci* 41, 579–590.
- Orlandini, V., Emiliani, G., Fondi, M., Maida, E., Perrin, E., Fani, R., 2014. Network Analysis of Plasmidomes: the *Azospirillum Brasilense* Sp245 Case. Hindawi Publishing Corporation 1–14.
- Oteino, N., Lally, R.D., Kiwanuka, S., Lloyd, A., Ryan, D., Germaine, K.J., et al., 2015. Plant growth promotion induced by phosphate solubilizing endophytic *Pseudomonas* isolates. *Front Microbiol* 6, 745.
- Ozturk, A., Caglar, O., Sahin, F., 2003. Yield response of wheat and barley to inoculation of plant growth promoting rhizobacteria at various levels of nitrogen fertilization. *J Plant Nutr Soil Sci* 166, 262–266. Available from: <https://doi.org/10.1002/jpln.200390038>.
- Parmar, P., Sindhu, S.S., 2013. Potassium solubilisation by rhizosphere Bacteria: influence of nutritional and environmental conditions. *J Microbial Res* 3, 25–31.
- Prathap, M., Ranjitha, K.B.D., 2015. A critical review on plant growth promoting rhizobacteria. *J Plant Pathol Microbiol* 6 (4), 1–4.
- Rathore, P., 2015. A review on approaches to develop plant growth promoting rhizobacteria. *Int J Recent Sci Res* 5 (2), 403–407.
- Richardson, A.E., 2001. Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. *Funct Plant Biol* 28, 897–906.
- Rivas, R., Peix, A., Mateos, P.F., Trujillo, M.E., Martínez-Molina, E., Velázquez, E., 2006. Biodiversity of populations of phosphate solubilizing rhizobia that nodulates chickpea in different spanish soils. *Plant and Soil* 287, 23–33.
- Russo, A., Carrozza, G.P., Vettori, L., Felici, C., Cinelli, F., Toffanin, A., 2012. Plant beneficial microbes and their application in plant biotechnology. In: Agbo, E.C. (Ed.), *Innovations in Biotechnology*. InTech University, Rijeka. Available from: <https://doi.org/10.5772/31466>.
- Saha, M., Sarkar, S., Sarkar, B., Sharma, B.K., Bhattacharjee, S., Tribedi, P., 2016. Microbial siderophores and their potential applications: a review. *Environ Sci Pollut Res* 23 (5), 3984–3999.
- Sahin, F., Cakmakci, R., Kanta, F., 2004. Sugar beet and barley yields in relation to inoculation with N₂-fixing and phosphate solubilizing bacteria. *Plant Soil* 265, 123–129. Available from: <https://doi.org/10.1007/s11104-005-0334-8>.
- Singh, L.P., Gill, S.S., Tuteja, N., 2011. Unraveling the role of fungal symbionts in plant abiotic stress tolerance. *Plant Signal Beh* 6, 175–191.
- Singh, M., Kumar, A., Singh, R., Pandey, K.D., 2017a. Endophytic bacteria: a new source of bioactive compounds. *3 Biotech* 7, 315.
- Singh, V.K., Singh, A.K., Kumar, A., 2017b. Disease management of tomato through PGPB: current trends and future perspective. *3 Biotech* 7 (4), 255.

- Tao, G.C., Tian, S.J., Cai, M.Y., Xie, G.H., 2008. Phosphate solubilizing and mineralizing abilities of bacteria isolated from soil. *Pedosphere* 18, 515–523.
- Uren, N.C., 2007. Types, amounts, and possible functions of compounds released into the rhizosphere by soil-grown plants. In: Pinton, R., Varanini, Z., Nannipieri, P., Raton, B. (Eds.), *The rhizosphere. Biochemistry and organic substances at the soil-plant interface*, second ed. CRC Press/Taylor & Francis Group, FL, pp. 1–21.
- van der Heijden, M.G.A., de Bruin, S., Luckerhoff, L., van Logtestijn, R.S., Schlaeppli, K., 2016. A widespread plant-fungalbacterial symbiosis promotes plant biodiversity, plant nutrition and seedling recruitment. *ISME J* 10, 389–399.
- Wolfe, B.E., Klironomos, J.N., 2005. Breaking new ground: soil communities and exotic plant invasion. *Bioscience* 55, 477.
- Yao, H.Y., Liu, Y.Y., Huang, C.Y., 2006. Effect of copper on phospholipids fatty acid composition of microbial communities in two red soils. *J Environ Sci* 18, 503–509.
- Zaidi, A., Khan, M.S., Ahemad, M., Oves, M., 2009. Plant growth promotion by phosphate solubilizing bacteria. *Acta Microbiol Immunol Hung* 56, 263–284.
- Zhang, J., Liu, J., Meng, L., Ma, Z., Tang, X., Cao, Y., et al., 2012. Isolation and characterization of plant growth-promoting rhizobacteria from wheat roots by wheat germ agglutinin labeled with fluorescein isothiocyanate. *J Microbiol* 50, 191–198. Available from: <https://doi.org/10.1007/s12275-012-1472-3>.

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Mechanisms of Plant-Microbe Interactions and its Significance for Sustainable Agriculture

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2.1 INTRODUCTION

Communication among the organism is one of the finest gifts of nature that played a key role in evolution and the complexity of life on Earth (Ma et al., 2016). Since the plants are unable to escape under adverse environmental conditions, they have evolved an array of biochemical and structural defense mechanisms against biotic and abiotic stresses. Indigenously, plants and microbes coexist in nature since long and their association either positive [interaction of the plants with the soil microbiota conferring stress tolerance (biotic and abiotic), and induce plant growth] or negative way (host-pathogen interactions leading to the disease development) may have significant impact on sustainable agriculture (Newton et al., 2010). To convey essential information required for growth and survival, plants release various signals/clues that make effective communication between plant and microbes. (Hückelhoven, 2007). Establishment of effective communication between plants and microbes may lead to develop several beneficial relationships between them viz., symbiotic, mutualistic, and commensalism relationships to boost the plant immunity (Badri et al., 2009). In same way, microbes viz., bacteria, fungi, etc. also secrete an array of chemical that are able to alter the composition of root exudates which may negatively affect the physiology of plants (Pritchard and Birch, 2011). The root system traditionally thought only to provide anchorage and conduction of nutrients and water. However, apart from the anchorage role, exudates secreted from roots allocates

carbon and micronutrients (sugars, amino acids, organic acids, and polymerized sugars) to the soil to tackle with pathogenic microbial neighbors and lure the beneficial one (Badri et al., 2009; Glick, 2014). In general exudates contain enzymes, water, ions, and an array of primary and secondary compounds that maintain the soil microbial community, and play an important role in plant growth and maintenance (Kumar et al., 2014). Shoot system also serves as a deck for these interactions by means of aromatic secondary metabolites compounds. Endophytic microbes associated with shoots have potential to synthesis secondary metabolite product of associated plants. In addition, plant-microbe association could be considered as novel candidate for bioremediation. Soil pollutants viz., heavy metals, surfactants, emulsifiers, and other complex organic and inorganic contaminants are some of the major causes of reduction in soil fertility and plant tissue damages (Santillo and Johnston, 2003). Phytosiderophores have played remarkable role in sequestration of heavy metals and soil bioremediation. Currently, a number of consortium based on plant-microbial associations have been used to mitigate the problem imposed by soil pollutant viz., heavy metal toxicity, pesticides residues and surfactants, emulsifiers, etc., in complex environments (Abhilash et al., 2012; Ma et al., 2016). The recent advancement in the field of molecular biology viz., proteomic, metabolomic, genomic, metagenomic, and transcriptomic approaches could elucidate the mechanism of plant-pathogen interaction at molecular levels (Kaul et al., 2016). In addition, how the pathogens are able to resist/ overcome the plant defense leading to the emergence of disease and new pathogenic species. The overall understanding of pathogen action and plant responses would enable development of disease control strategies, a challenging goal of mankind (Li et al., 2013). The climatic factors viz., temperature, light and C- flux significantly affect the plant-microbe association (Hua, 2013). In nature, physical, chemical, biological, and environmental signals are involved to regulate the plant-microbe association governing a complex biological activity including biofertilization, bioremediation, and biocontrol (Singh et al., 2017). Thus, the understanding of mechanism of association between the plants and their associated microbes with the ever-changing environment may give an idea about their relationships, either beneficial or detrimental and have significant impact of sustainable agriculture.

In this chapter, we provide an overview on significance of plants-microbe interaction and its mechanism with emphasis on pathogenesis and mutualism. In addition, their potential applications as biofertilizer,

rhizoremediation, and biocontrol agents along with the factors affecting plant-microbe interactions, and future research direction have been discussed.



2.2 CATALOGUING THE PLANT-MICROBE INTERACTION

Every organism on earth has to manage associations with its neighbors for their growth and survival. From very beginning, plant bodies are in the co-evolutionary fight for dominance with microbial community which provide an insight of the importance of its dynamic effect on agricultural systems (Jones and Dangel, 2006; Chisholm et al., 2006). In nature, plants make associations with neighboring plants and microflora for nutrient availability, assimilation, and exalt immune system. In general, plant-microbe communication has been categorized in three types viz., mutualism (associations with beneficial microbes), pathogenesis (interaction with some pathogenic microbes), and parasitism (cause collateral damage and obtained resources from their hosts) (Hückelhoven, 2007). These establishments form an array of interactions that may range from the negative to beneficial effects on both the host and invading microbes. Although, the pathogenic microbes are low in plant associative niche, but they also form a percentage in microbial population and significantly influence the economy of plant and their products. The molecular convergence of pathogen infection and plant immunity displays the plant-pathogen interactive venture from the perspective of both the organisms. The first level of the innate immune response system of plants responds initially to microbial associated molecules that may belong to the common class including nonpathogenic and virulent (Dodds and Rathjen, 2010). This interacting structure provides an extraordinary molecular insight of cell recognition, cell biology, and evolutionary processes across biological kingdoms which help in detailing the understanding of plant immune functions and suggest novel biotechnological approaches that will underpin the crop improvement and protection. In agricultural point of view, pathogenesis and mutualism describe important attributes of plant and microbe relationship and the understanding of these specific relationship mechanisms could play important role in sustainable agriculture (Li et al., 2013). Plant growth promoting rhizobacteria (PGPR) are widely

used as inoculants for biofertilization, rhizoremediation, and biocontrol agent. It reduces the use of chemical fertilizers and pesticides to a great extent which often pollute the environment.

2.2.1 A systemic perspective of plant-microbe interaction: Symbiosis verses pathogenesis

All plant-microbe interactions can have mutualistic or detrimental effects on the host during their attempts to obtain nutrients and environmental protection, irrespective of the physical associations in between which is needed for the success of the interaction (Soto et al., 2011). Widely accepted theory states that plant perceives both pathogenic as well as beneficial bacteria as intruders and thus mount the defense responses against them. But the successful interaction depends on the strategies and weapons used by the microbe to successfully infect the plant tissues while ability to evade or surpass the plant defense machinery was also a requirement (Soto et al., 2006). Lastly, the outcome of the plant-microbe interactions relies on the abilities of the host and microbe to reconcile their respective preference of mode of interaction, that is, from pathogenic to a mutual give-and-take process involving chemical signaling (Soto et al., 2009). Pathogenetic bacteria in the first place colonize the apoplast of plant tissues or the xylem that often involves the participation of hydrolytic enzymes and toxins while, mutualistic ones provide overall benefit to both partners based on nutrient exchange. In general, host pathogen recognition is mediated by two pathways viz., transmembrane pattern recognition receptors (PRRs) of plant recognized and respond to pathogen-associated molecular patterns (PAMPs) and R gene products such as polymorphic nuclear-binding and leucine-rich repeat proteins (NB-LRR). The primary recognition of pathogen effectors by R gene products stimulates the active defense responses (Dodds and Rathjen, 2010). The “zigzag” model of pathogen-plant interactions proposed by Jones and Dangl (2006) is widely accepted model deciphering the four different phase of host pathogen interaction. In the first phase, PRRs recognize PAMPs leads to inducing PAMP-triggered immunity (PTI) to overcome the further colonization and other microbial activity. In second phase, the microbes after entering inside the cell release the effector molecules that interfere with PTI and disturbed its functioning resulting in effector-triggered susceptibility (ETS). In thirds phase, some of the effectors were recognized by R proteins resulting in effector-triggered immunity (ETI) that induces hypersensitive response resulting in cell

death thereby protecting cell from microbial attack. Finally, in phase four, to reduce the ETI potential pathogen produced evolved effectors molecule/or modifying the previously recognized effectors. The recent molecular approaches revealed that the symbionts are act as “intelligent pathogens.” Global transcription analysis of *Lotus japonicus* (D’Antuono et al., 2008; Deguchi et al., 2007) and *Medicago truncatula* (Jones et al., 2008; Lohar et al., 2006) showed that the upregulation of the plant defense genes after inoculation with beneficial microbes and afterwards decline in their levels with nodule development too accord with the notion that a very fine line separates pathogen from symbionts (Djordjevic et al., 1987). The microbial gene that encode T3SSs (type III secretion systems) and T4SSs (type IV secretion systems) facilitate the effectors from the microbial cytoplasm to the plant cytoplasm leading to mutualism or pathogenesis. Genome sequencing of *Rhizobium* sp. NGR234, *Bradyrhizobium japonicum* USDA110 (Zehner et al., 2008; Süß et al., 2006), *Mesorhizobium loti* MAFF303099 (Hubber et al., 2004), and *M. loti* R7A (Hubber et al., 2004) revealed that the cluster of genes encode a T3SS (Pueppke and Broughton, 1999) governing the phenomenon of mutualism. The effectors release by pathogens suppress the plant innate immunity by inducing the PAMPs by altering the conformational and biochemical changes in host protein, RNA metabolism, kinases activity involved in plant defense (Block et al., 2008). The rhizobial effectors studied to date exhibited remarkable homology with proteins secreted by pathogens thus revealed the same kind of function to establish their respective interaction with plants. Till date, exact molecular mechanism of effectors during the establishment of symbiosis/pathogenesis in respect to various environmental factors are not clear (Deakin and Broughton, 2009). Schematic representations of plant-microbe interaction to establish the mutualistic and pathogenic association are described in Fig. 2.1.

2.2.2 Plant-microbe interaction: Biofertilizer

The symbiotic aspect of plant-microbe interaction has been played a remarkable role as a biofertilizer to boost the plant growth. Increasing nutrient availability for plants make PGPR participation effective in sustainable agriculture. In symbiosis, the bacteria utilize the inert nitrogen from the surrounding atmosphere and convert them in usable form (ammonium and nitrate) for the plants and obtain carbon source from the respective host plant. As per an estimate PGPR viz., *Rhizobium*,

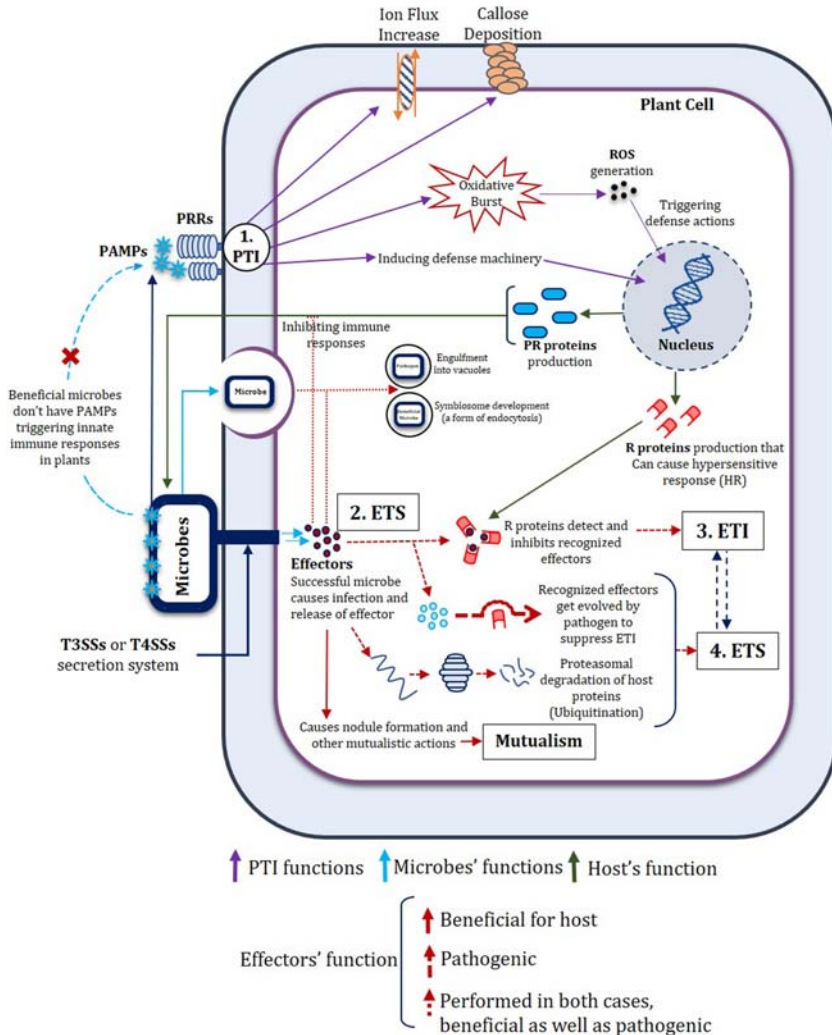


Figure 2.1 Representation of plant-microbe interaction in the view of mutualistic as well as pathogenic actions.

Azorhizobium, and *Sinorhizobium* genera counts for the 65% of the total nitrogen supply to agri-crops worldwide (Berrada and Fikri-Benbrahim 2014; Prasad et al., 2014). The most efficient biofertilizing bacterial strains belong to the genera *Rhizobium*, *Sinorhizobium*, *Mesorhizobium*, *Bradyrhizobium*, *Azorhizobium*, and *Allorhizobium*. Principally, the two genes cluster of genes viz., *nif* and *nod* governing the process of nitrogen

fixation. Molecular dissection of many bacteria led to identification of the genes on their chromosomes or symbiosis plasmid (*sym*), that mimic the rhizobial T3SS which is involved in the secretion of certain “symbiosis-forming proteins” (Ji and Dong, 2015; Almario et al., 2014). This machinery had been isolated from many plant beneficial bacterial strains including *Pseudomonas fluorescens*. Currently, a huge number of biofertilizer based on symbiotic relationship between plant and microbes have been widely used in agriculture as a sustainable alternative of synthetic fertilizer (Kumar et al., 2009). However, further research warrant to develop in vitro synergistic formulation of using microbial consortia under diverse stress condition (soil pH, presence of organic acids, stress factors, carbon source availability) to improve the fertility of soil and overall efficiency of biofertilization process.

2.2.3 Plant-microbe interactions: Rhizoremediation

The exhaustive increase in industrialization over the last century has led to impose the burden of anthropogenic chemicals viz., pesticides, solvents, metals, polycyclic aromatic hydrocarbons, and salt into the agricultural field (Huettermann et al., 2009). These anthropogenic hazardous chemical negatively affect the quality of soil and affect the crop productivity. Rhizoremediation is a process of degradation of pollutants by rhizomicrobial population of plants at contaminated site (Hao et al., 2014). Recently, plant-microbe interaction has been exploiting to remove such contaminant from soil that offers a cheaper, safer, and eco-friendly alternative of currently available methods. Kim et al. (2010) reported that citric acid and oxalic acid from *Echinochloa crusgalli* significantly enhanced both translocation and bioaccumulation of metals (Cd, Cu, and Pb), suggesting that organic acids can be considered natural chelating agents. Genoproteomic machinery of plants and their associated root microflora leads to their survival in the changing environment. The rhizomicrobial composition of the plant quantitatively linked to the composition of its root exudates that causes dynamic interaction between them. Root exudates induce the capability of host plants to adapt and survive under distressed conditions either by allelopathic functions (affecting the growth of rhizosphere microbes and other plants), or detoxification process (including adsorption, chelation, transformation, and inactivation of metals) (Luo et al., 2012). Since, the plant growth is directly or indirectly influenced by the soil pollutant, its removal of pollutant by means of natural

alternative could enhance the agricultural productivity. Table 2.1 summarizes the plant and microbes associated with removal of soil pollutant and plant growth promotion.

2.2.4 Plant-microbe interactions: Biocontrol agent

The use of biocontrol agent to prevent the plant disease is one of widely growing field of agroindustry in view of harmful effect of synthetic chemicals. In past few decades, this phenomenon has been widely used to control several plant diseases under the umbrella of biocontrol process. Microorganism viz., *Bacillus*, *Pseudomonas*, *Streptomyces* and *Trichoderma*, *Gliocladium*, and *Fusarium* are some of the most common bacterial and fungal genus widely used in preparation of different consortia used as biocontrol agent (Kumar et al., 2017; Kloepper et al., 2004; Bakker et al., 2014). In general, the biocontrol agent inhibits the growth and virulence potential of pathogenic organism via, niche exclusion, competition for nutrients, production of cell wall degrading enzymes viz. chitinase, toxic secondary metabolites and by induction of induced systemic resistance in the host plant. *Bacillus*, *Trichoderma*, and *Pseudomonas* strains either alone or in combination effectively protect the vegetable plants cucumber and tomato from pathogenic microorganism *Alternaria*, *fusarium*, and *Pythium* (Xu et al., 2014; Hao et al., 2014; Singh and Siddiqui, 2015; Kumar et al., 2014). Therefore, considerable attention has been paid by the researcher to develop microbial consortia that act synergistically to protect plant from disease as well as overcome the problem associated with synthetic chemicals. Table 2.2 summarizes the list of biocontrol agent and their mode of action against pathogenic microorganism and their associated crops.



2.3 FACTORS GOVERNING PLANT-MICROBE INTERACTIONS

Plant responds correspondingly in its interactive episode with microbes which is based on a number of stimuli factors viz., physical, chemical, and environmental. All the three factors play accordingly in a joint venture governing their own aspect to laid the successful interactive phenomena in between the plants and microbes. Physical signals deploy

Table 2.1 The plant and microbes associated with removal of heavy metals and plant growth promotion

Plant	Microbe	Heavy metal	Plant growth promoting traits	Reference
<i>Brassica napus</i> , <i>Solanum lycopersicum</i> , <i>Brassica juncea</i>	<i>Kluyvera ascorbata</i> SUD165, <i>Kluyvera ascorbate</i> SUD165/26	Zn, Ni, Pb	ACC deaminase and IAA	Burd et al. (2000)
<i>Brassica juncea</i>	<i>Enterobacter sp.</i> NBRI K28 and NBRI K28 SD1	Ni, Zn and Cr	ACC deaminase	
<i>Alnus firma</i>	<i>Bacillus thuringiensis</i> GDB-1	Cd, Pb, Cu, Zn, and As	IAA, ACC deaminase and siderophores, and mineral phosphate solubilization	Babu et al. (2015)
<i>Miscanthus sinensis</i>	<i>Pseudomonas koreensis</i> AGB-1	Cd, Pb, Cu, Zn, and As	IAA and ACC deaminase production	Babu et al. (2015)
<i>Solanum nigrum</i>	<i>Pseudomonas sp.</i> Lk9	Cd, Zn, Cu and Cr	Biosurfactants, siderophores, and organic acid producing	Chen et al. (2014)
<i>Polygonum pubescens</i>	<i>Rahnella sp.</i> JN6	Cd, Pb, Cu, and Zn	ACC deaminase	He et al. (2013)
<i>Sorghum bicolor</i> L.	<i>Bacillus sp.</i> SLS18	Cd and Mn	ACC deaminase and siderophore production	Luo et al. (2012)
<i>Sedum plumbizincicola</i>	<i>Bacillus pumilus</i> E2S2	Cd and Zn	IAA, siderophore, and ACC deaminase	Ma et al. (2015)
<i>Pteris vittata</i> and <i>P. Multifida</i>	<i>Bacillus sp.</i>	As	Enhance bioremediation	Zhu et al. (2014)
<i>Zea mays</i>	<i>Rahnella sp.</i> JN27	Cd	IAA, Siderophore, ACC deaminase, and solubilizing phosphate	Yuan et al. (2014)
<i>Vigna radiata</i>	<i>Exiguobacterium sp</i>	As	Solubilization of phosphate, production of indole-3-acetic acid (IAA) and exopolysaccharide (EPS)	Pandey and Bhatt (2016)

(Continued)

Table 2.1 (Continued)

Plant	Microbe	Heavy metal	Plant growth promoting traits	Reference
<i>Raphanus sativus</i>	<i>Bacillus</i> sp. CIK-516 and <i>Stenotrophomonas</i> sp. CIK-517Y	Ni	Indole acetic acid and 1-amino-cyclopropane-1-carboxylate deaminase potentials	Akhtar et al. (2018)
<i>Alyssum pintodasilvae</i>	<i>Arthrobacter nicotinovorans</i> SA40	Ni	IAA, P-solubilization, and siderophore	Cabello-Conejo et al. (2014)
<i>Carpobrotus rossii</i>	<i>Cedecea davisae</i> LCR1	Cd, Cu, and Zn	IAA, P-solubilization	Liu et al. (2015)
<i>Glycine max</i>	<i>Bradyrhizobium</i> sp. YL-6	Cd	IAA, ACC deaminase activity, siderophores, and P-solubilization	Guo and Chi (2014)
<i>Alnus firma</i>	<i>Bacillus</i> sp. MN3-4	Cd, Zn, Ni, Cu, Pb	Siderophores and IAA	Shin et al. (2012)
<i>Alyssum serpyllifolium</i>	<i>Pseudomonas</i> sp. A3R3	Ni	ACC deaminase, siderophores, and IAA and P-solubilization	Ma et al., 2011
<i>Salix caprea</i>	<i>Burkholderia</i> sp. RX232	Cd, Zn	ACC deaminase activity, siderophores	Kuffner et al. (2010)
<i>Medicago lupulina</i>	<i>Sinorhizobium meliloti</i> CCNWSX0020	Cu	IAA, ACC deaminase activity, and siderophore	Kong et al. (2015)
<i>Lens culinaris</i> var. <i>Malka</i>	<i>Rhizobium</i> sp. RL9	Cu, Cd, Cr, Ni, Pb, and Zn	IAA, siderophores, hydrogen cyanide, and ammonia	Wani and Khan (2013)
<i>Sorghum sudanense</i>	<i>Enterobacter</i> sp. K3-2	Cu	IAA, siderophores, ACC deaminase, Arginine decarboxylase	Li et al. (2016)

highly sensitive mechanosensory cell of plants to respond in various ways as in case of *Mimosa* and *Dionaea* species of plants (Johnson et al., 2014). Evidences conclude that on exposure with certain physical stimuli, specific pathways become activated within the plant (viz. calmodulin pathway) that mimic the microbial infestation aiding thereafter the resistance development (Stael et al., 2015; Pieterse et al., 2014). Microbes too use the physical aids for causing the infection, for example, penetration peg or hyphae that supporting the pathogen to use utilize shear mechanical force to breach further into the underlying tissue (*Colletotrichum* and *Magnaporthe grisea* species) (Wang and Qi, 2015). Plant responds to these stimuli via. membrane reorganization, cytoplasmic streaming, microtubule re-aggregation, and other mechanical responses that are observed in combination with molecular reactions in response to a microbial infection (Ueda et al., 2015). Chemical Signals let the chemical interactive play in between microbes and host which decipher the order of dialogs for the successful start and ending of the show. They also can be exploited to develop effective means via. genetic engineering technologies for the benefit of crops (Babar et al., 2016). Environmental factors conclude the abiotic and biotic stress factors that have significant effect on the aspect of interactive manner of plants and microbes. They directly regulate the molecular machinery inside the organisms that ultimately results in the changed interactive patterns between the two players. For example, nutrient deprived soil favors the siderophore producing microbes that play the beneficial role for the plants as it aids both of them to survive in the stress condition but also cause the bactericidal effect on the pathogens causing their diminished colonization (Ahmed and Holmström, 2014; Wackett, 2014). Environmental factors viz. agricultural practices, cropping systems, exposure to sunlight, temperature variation, and infestation with herbivorous agents serve as the main drivers in regulating plant-microbe interactions (Valentín-Vargas et al., 2014). Schematic representation of factors associated with plant-microbe interaction and their impacts are described in Fig. 2.2.

Table 2.2 The list of biocontrol agent and their mode of action against pathogenic microorganism and their associated crops

Plant	Disease	Pathogen	Biocontrol agent	Mode of action	Reference
Crops	Contamination diseases	<i>Aspergillus parasiticus</i> NRRL2999	<i>Bacillus subtilis</i> and <i>B. amyloliquefaciens</i>	• Inhibited <i>A. parasiticus</i> growth and aflatoxins B1 and G1 synthesis • Affected ergosterol synthesis • Suppressed fungal mitochondrial dehydrogenase activity • Paradoxical effect	Siahmoshteh et al. (2018)
<i>Cucumis sativus</i>	Anthracnose of cucumber	<i>Serratia marcescens</i> 90-166	<i>Colletotrichum orbiculare</i>	• Induced systemic resistance (ISR) • Reduction in internal root populations	Press et al. (2001)
<i>Cajanus cajan</i>	Wilt disease	<i>Bacillus</i> and <i>fluorescent pseudomonads</i> isolates	<i>Fusarium udum</i>	• Showing wilt disease complex • Isolates used for biocontrol of wilt disease complex	Siddiqui et al. (2005)
<i>Triticum aestivum</i>	Leaf blight of wheat	<i>Pseudomonas fluorescens</i> , <i>Azotobacter chroococcum</i>	<i>Alternaria triticina</i>	• <i>triticina</i> caused reduction in plant growth • Inoculation with <i>A. triticina</i> caused a significant reduction in photosynthetic pigments • Fly ash-amended soil caused significant increase in photosynthetic pigments	Siddiqui and Singh (2005)
<i>Oryza sativa</i>	Rice bacterial blight	<i>Pseudomonas fluorescens</i>	<i>Xanthomonas oryzae</i> <i>pv. oryzae</i>	• Disease intensity decreased • <i>P. fluorescens</i>-treated seeds did not show resistance to the pathogen • Increase in lignification and activities of peroxidase, phenylalanine ammonialyase and 4-coumarate: CoA ligase	Vidhyasekaran et al. (2001)
<i>Solanum lycopersicum</i>	Bacterial wilt of tomato	<i>Serratia</i> J2, <i>Pseudomonas</i> , <i>Bacillus</i> BB11	<i>Ralstonia solanacearum</i>	• Tested strains decreased disease incidence and increased yield • Biocontrol efficiencies increases	Guo et al. (2004)

<i>Solanum lycopersicum</i>	Bacterial speck of tomato	<i>Azospirillum brasilense</i>	<i>Pseudomonas syringae</i> pv. <i>tomato</i>	• Inoculation of bacterial strains resulted in a reduction of the pathogen population in the rhizosphere • Prevention of bacterial speck disease development • Improved plant growth • Decreased disease severity • Enhanced plant development	Bashan and de-Bashan (2002)
<i>rassica oleracea</i>	Black rot	<i>Bacillus. cereus</i> , <i>B. lentimorbus</i> , <i>B. pumilus</i>	<i>Xanthomonas compestris</i> pv. <i>compestris</i>	• High disease severity • Treated seeds with strain result in reduction of black rot	Massomo et al. (2004)
<i>Solanum lycopersicum</i>	Damping-off of tomato	<i>Bacillus subtilis</i> <i>Burkholderia cepacia</i>	<i>Rhizoctonia solani</i>	• Control of damping-off caused by <i>R. solani</i> by RB + BY2 • Both agents gave better plant protection • Reduction of plant infection	Szczech and Shoda (2004)
<i>Pennisetum glaucum</i>	Downy mildew	<i>Pseudomonas fluorescens</i>	<i>Sclerospora graminicola</i>	• Reduced disease severity and promoted growth • Induced resistances against downy mildew	Raj et al. (2004)
<i>Cucumis melo</i>	Fusarial Wilt	<i>Pseudomonas putida</i>	<i>Fusarium oxysporum</i> f. sp. <i>melonis</i>	• Reduced wilt severity caused by <i>F. oxysporum</i>	Bora et al. (2004)
<i>Pennisetum glaucum</i>	Downy Mildew	<i>Bacillus pumilus</i>	<i>Sclerospora graminicola</i>	• Prominent enhancement in growth • PGPR strain INR7 suppressed downy mildew effectively • Metalaxyl resulted in the highest protection against downy mildew	Raj et al. (2003)

(Continued)

Table 2.2 (Continued)

Plant	Disease	Pathogen	Biocontrol agent	Mode of action	Reference
<i>Cicer arietinum</i> L.; <i>Cajanus cajan</i> L.	Fusarium wilt disease	<i>Pseudomonas aeruginosa</i> PNA 1	<i>Fusarium udum</i> , <i>Fusarium oxysporum</i> f. sp. <i>ciceris</i>	• Inoculation with strain PNA1 significantly reduced the incidence of <i>fusarium</i> wilt • Root colonization measured using a <i>lacZ</i>-marked strain of PNA1 • Strain PNA1 produced two phenazine antibiotics, phenazine-1-carboxylic acid and oxychlororaphin,	Anjaiah et al. (2003)
<i>Saccharum officinarum</i>	Red rot disease	<i>Pseudomonas fluorescens</i>	<i>Colletotrichum Falactum</i>	• Strains of fluorescent <i>Pseudomonas</i> spp. induced systemic resistance • <i>Pseudomonas</i> strains reduced red rot disease intensity • Less pathogen induced invertase enzyme activity	Viswanathan and Samiyappan (2002)
<i>Arachis hypogaea</i>	Crown rot and wilt diseases	<i>Bacillus subtilis</i> AF1	<i>Aspergillus niger</i> , <i>Fusarium udum</i>	• Increase in cell numbers of introduced <i>B. subtilis</i> AF 1	Manjula and Podile (2001)
<i>Oryza sativa</i>	Sheath blight disease	<i>Pseudomonas fluorescens</i> strains Pf1, FP7	<i>Cnaphalocrocis medinalis</i>	• Reduction in leafhopper incidence • Reduced larval and pupal weight, increased larval mortality and • incidence of malformed adults under in vitro	Commare et al. (2002)
<i>Cucumis sativus</i>	Root rot	<i>P. corrugate</i> , <i>P. aureofaciens</i>	<i>Pythium aphanidermatum</i>	• Induced systemic resistance	Chen et al. (1999)
<i>Pinus taeda</i>	Fusiform rust	<i>B. pumilus</i> SE34 <i>S. marsecens</i> 90-166	<i>Cronartium quercuum</i> f. sp. <i>fusiforme</i>	• Induce systemic protection • Reducing the number of galls	Enebak and Carey (2000)

<i>Oryza sativa</i> L.	Brown spot	<i>Bipolaris oryzae</i>	<i>Trichoderma harzianum</i>	• Reduced the disease severity • Reduce disease incidence • Increase in the total photosynthetic pigments (chlorophyll a and b and carotenoids)	Abdel-Fattah et al. (2007)
<i>Solanum lycopersicum</i>	Wilt disease	<i>Ralstonia solanacearum</i>	<i>Acinetobacter</i> sp. Xa6; <i>Enterobacter</i> sp. Xy3	• Higher biocontrol efficacy and plant yield increase	Xue et al. (2009)

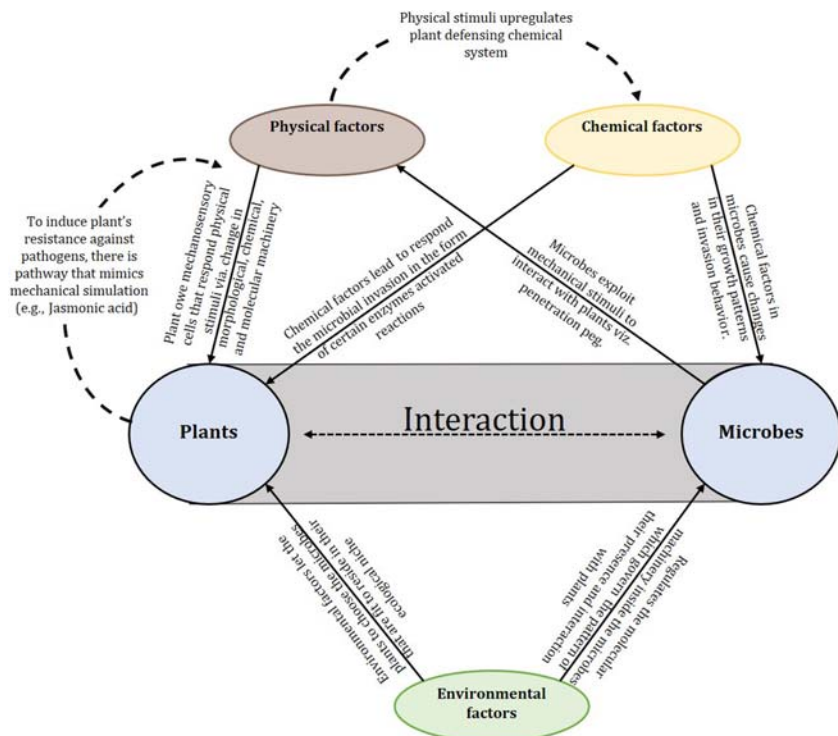


Figure 2.2 Representation of factors associated with plant-microbe interaction and their impacts on interaction.



2.4 APPLICATIONS OF PLANT-MICROBE INTERACTIONS

Although the potential use of plant-microbe interactions has been recommended as a means to maximize the economic returns of agribusiness, still there are widespread concerns regarding the use of plant-microbe chemical signaling and their interactive coordination in bioremediation of soil pollutants. Recent advancements in several meta- and omics-technologies provide the moment to exploit proteomic, metabolomic, genomic, metagenomic, and transcriptomic approaches to identify traits that maximize the benefits of modern agricultural practices and manipulate the plant-microbe relations toward the sustainable agriculture. Microbes are the causes of both beneficial (bioremediation, biofertilization, and biocontrol) and pathogenic effects (associated with famine and

poverty caused by the plant diseases) in the plant body. But the pathogenesis is just a functional phase of the microorganism occurred at the propagating phase of their life cycle which happens at the expense of the host. Hence, modern agriculture practices and increasing awareness about plant-microbe interactions tend to focus more on the development of breeding or crop-protection schemes than making the attempt to eliminate the potential pathogens as making it more effective, or sustainable (Li et al., 2013; Newton et al., 2010). Treatment of roots of young plants with several PGPR strains as biofertilization process, inoculating plants with a number of bacterial and fungal species as biocontrol methods, plant-microbe interactive consumption of organic pollutants as rhizoremediating phenomena, are some of the recent progressions made in the way of sustainable agriculture and environment revitalization having a low-input biotechnology prospects. Utilization of whole organisms, that is, bacteria and fungus, for the improvement of crop yield, became the method of old days, while the recent methodology lends molecular and sub-molecular units of the microbes (Bourras et al., 2015). Along with the betterment of crop yields and help in sustainable agriculture, mankind also gets served via. gaining a variety of medical and economic benefits that's coming from the overexpression of the secondary metabolites such as alkaloids, volatile oils, and antibiotics by the use of the molecular pharming techniques (Maag et al., 2015). Hence, molecular insights of plant-microbe interactions bring the advancement in agriculture practices along with the ecological gains. Moreover, future manipulation of desired plant-microbe interactive conquest with genetic engineering approaches should facilitate more advance and organic agro-practices ensuring complete utilization of beneficial microbial ecological interactions for additional benefits.



2.5 CONCLUSION AND FUTURE PERSPECTIVES

Plant-microbe interactions significantly influence the individual growth, community assembly, and biogeochemical cycling in terrestrial ecosystems. In addition, plant-microbe interaction has been played a vital role to design the biocontrol, biofertilizer, and bioremediation agents in sustainable agriculture. Although, plethora of literature are available on plant-microbe interaction, the exact molecular mechanisms underlying

the functioning the genes responsible for the transportation of signal molecules across the cell membranes at the biotrophic level especially during the mutualistic and pathogenic interactions are lacking. Therefore, understanding the basis of the relationships between plant and microbe may provide key insights of biological phenomenon to boost plant health, disease control, and risk management. Climatic factors, along with the various biological factors play crucial role in the plant-microbes interaction. Thus, the future research must be focused on deciphering the mechanism of plant-microbe interactions various biotic and abiotic environmental conditions including host-specific factors with large numbers of replicates. The understanding of plant-microbe interactions mechanism could be helpful in minimizing disease incidence, pathogen outbreaks, enhancing biodiversity, plant productivity, and maximize the profitable functions in sustainable agriculture.

REFERENCES

- Abdel-Fattah, G.M., Shabana, Y.M., Ismail, A.E., Rashad, Y.M., 2007. *Trichoderma harzianum*: a biocontrol agent against *Bipolaris oryzae*. *Mycopathologia* 164 (2), 81–89.
- Abhilash, P.C., Powell, J.R., Singh, H.B., Singh, B.K., 2012. Plant–microbe interactions: novel applications for exploitation in multipurpose remediation technologies. *Trends Biotechnol* 30 (8), 416–420.
- Ahmed, E., Holmström, S.J., 2014. Siderophores in environmental research: roles and applications. *Microb Biotechnol* 7 (3), 196–208.
- Akhtar, M.J., Ullah, S., Ahmad, I., Rauf, A., Nadeem, S.M., Khan, M.Y., et al., 2018. Nickel phytoextraction through bacterial inoculation in *Raphanus sativus*. *Chemosphere* 190, 234–242.
- Almario, J., Gobbin, D., Défago, G., Moënné-Loccoz, Y., Rezzonico, F., 2014. Prevalence of type III secretion system in effective biocontrol pseudomonads. *Res Microbiol* 165 (4), 300–304.
- Anjaiah, V., Cornelis, P., Koedam, N., 2003. Effect of genotype and root colonization in biological control of fusarium wilts in pigeonpea and chickpea by *Pseudomonas aeruginosa* PNA1. *Can J Microbiol* 49 (2), 85–91.
- Babar, M.M., Khan, S.F., Zargaham, M.K., Gul, A., 2016. Plant-microbe interactions: a molecular approach. In: Hakeem, K., Akhtar, M. (Eds.), *Plant, Soil and Microbes*. Springer, Cham.
- Babu, A.G., Shea, P.J., Sudhakar, D., Jung, I.B., Oh, B.T., 2015. Potential use of *Pseudomonas koreensis* AGB-1 in association with *Miscanthus sinensis* to remediate heavy metal (loid)-contaminated mining site soil. *J Environ Manage* 151, 160–166.
- Badri, D.V., Weir, T.L., van der Lelie, D., Vivanco, J.M., 2009. Rhizosphere chemical dialogues: plant–microbe interactions. *Curr Opin Biotechnol* 20 (6), 642–650.
- Bakker, P.A., Ran, L., Mercado-Blanco, J., 2014. Rhizobacterial salicylate production provokes headaches!. *Plant Soil* 382 (1–2), 1–16.
- Bashan, Y., de-Bashan, L.E., 2002. Protection of tomato seedlings against infection by *Pseudomonas syringae* pv. tomato by using the plant growth-promoting bacterium *Azospirillum brasilense*. *Appl Environ Microbiol* 68 (6), 2637–2643.

- Berrada, H., Fikri-Benbrahim, K., 2014. Taxonomy of the Rhizobia: current perspectives. *Microbiol Res J* 4, 616–639.
- Block, A., Li, G., Fu, Z.Q., Alfano, J.R., 2008. Phytopathogen type III effector weaponry and their plant targets. *Curr Opin Plant Biol* 11 (4), 396–403.
- Bora, T., Özaktan, H., Göre, E., Aslan, E., 2004. Biological control of *Fusarium oxysporum* f. sp. melonis by wettable powder formulations of the two strains of *Pseudomonas putida*. *J Phytopathology* 152 (8–9), 471–475.
- Bourras, S., Rouxel, T., Meyer, M., 2015. *Agrobacterium tumefaciens* gene transfer: how a plant pathogen hacks the nuclei of plant and nonplant organisms. *Phytopathology* 105 (10), 1288–1301.
- Burd, G.I., Dixon, D.G., Glick, B.R., 2000. Plant growth-promoting bacteria that decrease heavy metal toxicity in plants. *Can. J. Microbiol.* 46 (3), 237–245.
- Cabello-Conejo, M.I., Becerra-Castro, C., Prieto-Fernández, A., Monterroso, C., Saavedra-Ferro, A., Mench, M., et al., 2014. Rhizobacterial inoculants can improve nickel phytoextraction by the hyperaccumulator *Alyssum pintodasilvae*. *Plant Soil* 379 (1–2), 35–50.
- Chen, C., Bélanger, R.R., Benhamou, N., Paulitz, T.C., 1999. Role of salicylic acid in systemic resistance induced by *Pseudomonas* spp. against *Pythium aphanidermatum* in cucumber roots. *Euro J Plant Pathol* 105 (5), 477–486.
- Chen, L., Luo, S., Li, X., Wan, Y., Chen, J., Liu, C., 2014. Interaction of Cd-hyperaccumulator *Solanum nigrum* L. and functional endophyte *Pseudomonas* sp. Lk9 on soil heavy metals uptake. *Soil Biol Biochem* 68, 300–308.
- Chisholm, S.T., Coaker, G., Day, B., Staskawicz, B.J., 2006. Host-microbe interactions: shaping the evolution of the plant immune response. *Cell* 124 (4), 803–814.
- Commare, R.R., Nandakumar, R., Kandan, A., Suresh, S., Bharathi, M., Raguchander, T., et al., 2002. *Pseudomonas fluorescens* based bio-formulation for the management of sheath blight disease and leaffolder insect in rice. *Crop Prot* 21 (8), 671–677.
- D'Antuono, A.L., Ott, T., Krusell, L., Voroshilova, V., Ugalde, R.A., Udvardi, M., et al., 2008. Defects in rhizobial cyclic glucan and lipopolysaccharide synthesis alter legume gene expression during nodule development. *Mol Plant Microbe Interact* 21 (1), 50–60.
- Deakin, W.J., Broughton, W.J., 2009. Symbiotic use of pathogenic strategies: rhizobial protein secretion systems. *Nat Rev Microbiol* 7 (4), 312.
- Deguchi, Y., Banba, M., Shimoda, Y., Chechetka, S.A., Suzuri, R., Okusako, Y., et al., 2007. Transcriptome profiling of *Lotus japonicus* roots during arbuscular mycorrhiza development and comparison with that of nodulation. *DNA Res* 14 (3), 117–133.
- Djordjevic, M.A., Gabriel, D.W., Rolfe, B.G., 1987. Rhizobium—the refined parasite of legumes. *Annu Rev Phytopathol* 25 (1), 145–168.
- Dodds, P.N., Rathjen, J.P., 2010. Plant immunity: towards an integrated view of plant–pathogen interactions. *Nat Rev Genet* 11 (8), 539.
- Enebak, S.A., Carey, W.A., 2000. Evidence for induced systemic protection to fusiform rust in loblolly pine by plant growth-promoting rhizobacteria. *Plant Dis* 84 (3), 306–308.
- Glick, B.R., 2014. Bacteria with ACC deaminase can promote plant growth and help to feed the world. *Microb Res* 169, 30–39.
- Guo, J., Chi, J., 2014. Effect of Cd-tolerant plant growth-promoting rhizobium on plant growth and Cd uptake by *Lolium multiflorum* Lam. and *Glycine max* (L.) Merr. in Cd-contaminated soil. *Plant Soil* 375 (1–2), 205–214.
- Guo, J.H., Qi, H.Y., Guo, Y.H., Ge, H.L., Gong, L.Y., Zhang, L.X., et al., 2004. Biocontrol of tomato wilt by plant growth-promoting rhizobacteria. *Biol Control* 29 (1), 66–72.

- Hao, K., Liu, J.Y., Ling, F., Liu, X.L., Lu, L., Xia, L., et al., 2014. Effects of dietary administration of *Shewanella haliotis* D4, *Bacillus cereus* D7 and *Aeromonas bivalvium* D15, single or combined, on the growth, innate immunity and disease resistance of shrimp, *Litopenaeus vannamei*. *Aquaculture* 428, 141–149.
- He, H., Ye, Z., Yang, D., Yan, J., Xiao, L., Zhong, T., et al., 2013. Characterization of endophytic *Rahnella* sp. JN6 from *Polygonum pubescens* and its potential in promoting growth and Cd, Pb, Zn uptake by *Brassica napus*. *Chemosphere* 90 (6), 1960–1965.
- Hua, J., 2013. Modulation of plant immunity by light, circadian rhythm, and temperature. *Curr Opin Plant Biol* 16 (4), 406–413.
- Hubber, A., Vergunst, A.C., Sullivan, J.T., Hooykaas, P.J., Ronson, C.W., 2004. Symbiotic phenotypes and translocated effector proteins of the *Mesorhizobium loti* strain R7A VirB/D4 type IV secretion system. *Mol. Microbiol.* 54 (2), 561–574.
- Hückelhoven, R., 2007. Transport and secretion in plant–microbe interactions. *Curr Opin Plant Biol* 10 (6), 573–579.
- Huettermann, A., Oriquiriza, L.J., Agaba, H., 2009. Application of superabsorbent polymers for improving the ecological chemistry of degraded or polluted lands. *CLEAN—Soil, Air, Water* 37 (7), 517–526.
- Ji, H., Dong, H., 2015. Key steps in type III secretion system (T3SS) towards translocon assembly with potential sensor at plant plasma membrane. *Mol Plant Pathol* 16 (7), 762–773.
- Johnson, K., Narasimhan, G., Krishnan, C., 2014. *Mimosa pudica* Linn—a shyness princess: a review of its plant movement, active constituents, uses and pharmacological activity. *Int J Pharm Sci Res* 5 (12), 5104.
- Jones, J.D., Dangl, J.L., 2006. The plant immune system. *Nature* 444 (7117), 323.
- Jones, K.M., Sharopova, N., Lohar, D.P., Zhang, J.Q., VandenBosch, K.A., Walker, G.C., 2008. Differential response of the plant *Medicago truncatula* to its symbiont *Sinorhizobium meliloti* or an exopolysaccharide-deficient mutant. *Proc Natl Acad Sci* 105 (2), 704–709.
- Kaul, S., Sharma, T., Dhar, M.K., 2016. “Omics” Tools for Better Understanding the Plant–Endophyte Interactions. *Front Plant Sci* 7, 955.
- Kim, S., Lim, H., Lee, I., 2010. Enhanced heavy metal phytoextraction by *Echinochloa crus-galli* using root exudates. *J Biosci Bioeng* 109 (1), 47–50.
- Kloepper, J.W., Ryu, C.M., Zhang, S., 2004. Induced systemic resistance and promotion of plant growth by *Bacillus* spp. *Phytopathology* 94 (11), 1259–1266.
- Kong, Z., Glick, B.R., Duan, J., Ding, S., Tian, J., McConkey, B.J., et al., 2015. Effects of 1-aminocyclopropane-1-carboxylate (ACC) deaminase-overproducing *Sinorhizobium meliloti* on plant growth and copper tolerance of *Medicago lupulina*. *Plant Soil* 391 (1–2), 383–398.
- Kuffner, M., De Maria, S., Puschenreiter, M., Fallmann, K., Wieshammer, G., Gorfer, M., et al., 2010. Culturable bacteria from Zn- and Cd-accumulating *Salix caprea* with differential effects on plant growth and heavy metal availability. *J Appl Microbiol* 108 (4), 1471–1484.
- Kumar, A., Verma, H., Singh, V.K., Singh, P.P., Singh, S.K., Ansari, W.A., et al., 2017. Role of *Pseudomonas* sp. in sustainable agriculture and disease management. *Agriculturally Important Microbes for Sustainable Agriculture*. Springer, Singapore, pp. 195–215.
- Kumar, C.A., Narinder, S., Daljeet, S., 2014. Exploitation of indigenous strains of *Trichoderma* and *Pseudomonas fluorescens* for the control of damping-off in chilli. *Plant Dis Res* 30 (1), 6–10.

- Kumar, S., Pandey, P., Maheshwari, D.K., 2009. Reduction in dose of chemical fertilizers and growth enhancement of sesame (*Sesamum indicum* L.) with application of rhizospheric competent *Pseudomonas aeruginosa* LES4. *Eur J Soil Biol* 45 (4), 334–340.
- Li, Y., Huang, F., Lu, Y., Shi, Y., Zhang, M., Fan, J., et al., 2013. Mechanism of plant–microbe interaction and its utilization in disease-resistance breeding for modern agriculture. *Physiol Mol Plant Pathol* 83, 51–58.
- Li, Y., Wang, Q., Wang, L., He, L.Y., Sheng, X.F., 2016. Increased growth and root Cu accumulation of *Sorghum sudanense* by endophytic enterobacter sp. K3-2: implications for *Sorghum sudanense* biomass production and phytostabilization. *Ecotoxicol Environ Saf* 124, 163–168.
- Liu, W., Wang, Q., Wang, B., Hou, J., Luo, Y., Tang, C., et al., 2015. Plant growth-promoting rhizobacteria enhance the growth and Cd uptake of *Sedum plumbizincicola* in a Cd-contaminated soil. *J Soils Sediments* 15 (5), 1191–1199.
- Lohar, D.P., Sharopova, N., Endre, G., Penuela, S., Samac, D., Town, C., et al., 2006. Transcript analysis of early nodulation events in *Medicago truncatula*. *Plant Physiol* 140 (1), 221–234.
- Luo, S., Xu, T., Chen, L., Chen, J., Rao, C., Xiao, X., et al., 2012. Endophyte-assisted promotion of biomass production and metal-uptake of energy crop sweet sorghum by plant-growth-promoting endophyte *Bacillus* sp. SLS18. *Appl Microbiol Biotechnol* 93 (4), 1745–1753.
- Ma, Y., Oliveira, R.S., Nai, F., Rajkumar, M., Luo, Y., Rocha, I., et al., 2015. The hyperaccumulator *Sedum plumbizincicola* harbors metal-resistant endophytic bacteria that improve its phytoextraction capacity in multi-metal contaminated soil. *J Environ Manage* 156, 62–69.
- Ma, Y., Oliveira, R.S., Freitas, H., Zhang, C., 2016. Biochemical and molecular mechanisms of plant-microbe-metal interactions: relevance for phytoremediation. *Front Plant Sci* 7, 918.
- Ma, Y., Rajkumar, M., Luo, Y., Freitas, H., 2011. Inoculation of endophytic bacteria on host and non-host plants—effects on plant growth and Ni uptake. *J Hazard Mater* 195, 230–237.
- Maag, D., Erb, M., Köllner, T.G., Gershenzon, J., 2015. Defensive weapons and defense signals in plants: some metabolites serve both roles. *Bioessays* 37 (2), 167–174.
- Manjula, K., Podile, A.R., 2001. Chitin-supplemented formulations improve biocontrol and plant growth promoting efficiency of *Bacillus subtilis* AF 1. *Can J Microbiol* 47 (7), 618–625.
- Massomo, S.M.S., Mortensen, C.N., Mabagala, R.B., Newman, M.A., Hockenhull, J., 2004. Biological control of black rot (*Xanthomonas campestris* pv. *campestris*) of cabbage in Tanzania with *Bacillus* strains. *J Phytopathology* 152 (2), 98–105.
- Newton, A.C., Fitt, B.D., Atkins, S.D., Walters, D.R., Daniell, T.J., 2010. Pathogenesis, parasitism and mutualism in the trophic space of microbe–plant interactions. *Trends Microbiol* 18 (8), 365–373.
- Pandey, N., Bhatt, R., 2016. Role of soil associated *Exiguobacterium* in reducing arsenic toxicity and promoting plant growth in *Vigna radiata*. *Eur J Soil Biol* 75, 142–150.
- Pieterse, C.M.J., Zamioudis, C., Does, D.V., Van Wees, S.C.M., 2014. Signalling Networks Involved in Induced Resistance. *Induced Resistance for Plant Defense* [Internet]. John Wiley & Sons, Ltd, pp. 58–80.
- Prasad, R., Kumar, M., Varma, A., 2014. Role of PGPR in Soil Fertility and Plant Health. *Plant-Growth-Promoting Rhizobacteria (PGPR) and Medicinal Plants* [Internet]. Springer International Publishing, pp. 247–260.
- Press, C.M., Loper, J.E., Kloepper, J.W., 2001. Role of iron in rhizobacteria-mediated induced systemic resistance of cucumber. *Phytopathology* 91 (6), 593–598.

- Pritchard, L., Birch, P., 2011. A systems biology perspective on plant–microbe interactions: biochemical and structural targets of pathogen effectors. *Plant Sci* 180 (4), 584–603.
- Pueppke, S.G., Broughton, W.J., 1999. *Rhizobium* sp. strain NGR234 and *R. fredii* USDA257 share exceptionally broad, nested host ranges. *Mol Plant Microbe Interact* 12 (4), 293–318.
- Raj, S.N., Chaluvajuru, G., Amruthesh, K.N., Shetty, H.S., Reddy, M.S., Kloepper, J.W., 2003. Induction of growth promotion and resistance against downy mildew on pearl millet (*Pennisetum glaucum*) by rhizobacteria. *Plant Dis* 87 (4), 380–384.
- Raj, S.N., Shetty, N.P., Shetty, H.S., 2004. Seed bio-priming with *Pseudomonas fluorescens* isolates enhances growth of pearl millet plants and induces resistance against downy mildew. *Int J Pest Manage* 50 (1), 41–48.
- Santillo, D., Johnston, P., 2003. Playing with fire: the global threat presented by brominated flame retardants justifies urgent substitution. *Environ Int* 29 (6), 725–734.
- Shin, M.N., Shim, J., You, Y., Myung, H., Bang, K.S., Cho, M., et al., 2012. Characterization of lead resistant endophytic *Bacillus* sp. MN3-4 and its potential for promoting lead accumulation in metal hyperaccumulator *Alnus firma*. *J Hazard Mater* 199, 314–320.
- Siahmoshteh, F., Hamidi-Esfahani, Z., Spadaro, D., Shams-Ghahfarokhi, M., Razzaghi-Abyaneh, M., 2017. Unraveling the mode of antifungal action of *Bacillus subtilis* and *Bacillus amyloliquefaciens* as potential biocontrol agents against aflatoxigenic *Aspergillus parasiticus*. *Food Control* 89, 300–307. Elsevier BV.
- Siddiqui, S., Siddiqui, Z.A., Ahmad, I., 2005. Evaluation of fluorescent *Pseudomonas* and *Bacillus* isolates for the biocontrol of a wilt disease complex of pigeonpea. *World J Microbiol Biotechnol* 21 (5), 729–732.
- Siddiqui, Z.A., Singh, L.P., 2005. Effects of fly ash and soil micro-organisms on plant growth, photosynthetic pigments and leaf blight of wheat. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* 112 (2), 146–155.
- Singh, N., Siddiqui, Z.A., 2015. Effects of *Bacillus subtilis*, *Pseudomonas fluorescens* and *Aspergillus awamori* on the wilt-leaf spot disease complex of tomato. *Phytoparasitica* 43 (1), 61–75.
- Singh, V.K., Singh, A.K., Kumar, A., 2017. Disease management of tomato through PGPB: current trends and future perspective. *3 Biotech* 7 (4), 255.
- Soto, M., Nogales, J., Pérez-Mendoza, D., Gallegos, M.T., Olivares, J., Sanjuán, J., 2011. Pathogenic and mutualistic plant–bacteria interactions: ever increasing similarities. *Open Life Sci* 6 (6), 911–917.
- Soto, M.J., Sanjuan, J., Olivares, J., 2006. Rhizobia and plant-pathogenic bacteria: common infection weapons. *Microbiology* 152 (11), 3167–3174.
- Soto, M.J., Domínguez-Ferreras, A., Pérez-Mendoza, D., Sanjuán, J., Olivares, J., 2009. Mutualism versus pathogenesis: the give-and-take in plant–bacteria interactions. *Cell Microbiol* 11 (3), 381–388.
- Stael, S., Kmiecik, P., Willems, P., Van Der Kelen, K., Coll, N.S., Teige, M., et al., 2015. Plant innate immunity—sunny side up? *Trends Plant Sci* 20 (1), 3–11.
- Süß, C., Hempel, J., Zehner, S., Krause, A., Patschkowski, T., Göttfert, M., 2006. Identification of genistein-inducible and type III-secreted proteins of *Bradyrhizobium japonicum*. *J Biotechnol* 126 (1), 69–77.
- Szczzech, M., Shoda, M., 2004. Biocontrol of Rhizoctonia Damping-off of Tomato by *Bacillus subtilis* combined with *Burkholderia cepacia*. *J Phytopathol* 152 (10), 549–556.
- Ueda, H., Tamura, K., Hara-Nishimura, I., 2015. Functions of plant-specific myosin XI: from intracellular motility to plant postures. *Curr Opin Plant Biol* 28, 30–38.

- Valentín-Vargas, A., Root, R.A., Neilson, J.W., Chorover, J., Maier, R.M., 2014. Environmental factors influencing the structural dynamics of soil microbial communities during assisted phytostabilization of acid-generating mine tailings: a mesocosm experiment. *Sci Total Environ* 500, 314–324.
- Vidhyasekaran, P., Kamala, N., Ramanathan, A., Rajappan, K., Paranidharan, V., Velazhahan, R., 2001. Induction of systemic resistance by *Pseudomonas fluorescens* Pfl against *Xanthomonas oryzae* pv. *Oryzae* in rice leaves. *Phytoparasitica* 29 (2), 155.
- Viswanathan, R., Samiyappan, R., 2002. Induced systemic resistance by fluorescent pseudomonads against red rot disease of sugarcane caused by *Colletotrichum falcatum*. *Crop Prot* 21 (1), 1–10.
- Wackett, L.P., 2014. Antibiosis in the environment. *Environ Microbiol Rep* 6 (5), 532–533.
- Wang, L.J., Qi, X., 2015. Metabolomics research of quantitative disease resistance against barley leaf rust. In: Hakeem, K., Akhtar, M. (Eds.), *Plant Metabolomics*. Springer, Dordrecht.
- Wani, P.A., Khan, M.S., 2013. Nickel detoxification and plant growth promotion by multi metal resistant plant growth promoting *Rhizobium* species RL9. *Bull. Environ. Contam. Toxicol.* 91 (1), 117–124.
- Xu, Z., Zhang, R., Wang, D., Qiu, M., Feng, H., Zhang, N., et al., 2014. Enhanced control of cucumber wilt disease by *Bacillus amyloliquefaciens* SQR9 by altering the regulation of its DegU phosphorylation. *Appl Environ Microbiol* 80 (9), 2941–2950.
- Xue, Q.Y., Chen, Y., Li, S.M., Chen, L.F., Ding, G.C., Guo, D.W., et al., 2009. Evaluation of the strains of *Acinetobacter* and *Enterobacter* as potential biocontrol agents against *Ralstonia* wilt of tomato. *Biol Control* 48 (3), 252–258.
- Yuan, M., He, H., Xiao, L., Zhong, T., Liu, H., Li, S., et al., 2014. Enhancement of Cd phytoextraction by two *Amaranthus* species with endophytic *Rhizobium* sp. JN27. *Chemosphere* 103, 99–104.
- Zehner, S., Schober, G., Wenzel, M., Lang, K., Göttfert, M., 2008. Expression of the *Bradyrhizobium japonicum* type III secretion system in legume nodules and analysis of the associated *tsx* promoter. *Mol Plant Microbe Interact* 21 (8), 1087–1093.
- Zhu, L.J., Guan, D.X., Luo, J., Rathinasabapathi, B., Ma, L.Q., 2014. Characterization of arsenic-resistant endophytic bacteria from hyperaccumulators *Pteris vittata* and *Pteris multifida*. *Chemosphere* 113, 9–16.

FURTHER READING

- Bais, H.P., Weir, T.L., Perry, L.G., Gilroy, S., Vivanco, J.M., 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. *Annu Rev Plant Biol* 57, 233–266.
- Gomes, D.F., Ormeño-Orrillo, E., Hungria, M., 2015. Biodiversity, symbiotic efficiency, and genomics of *Rhizobium tropici* and related species. *Biol. Nitrogen Fixation* 747–756.
- Krause, A., Doerfel, A., Göttfert, M., 2002. Mutational and transcriptional analysis of the type III secretion system of *Bradyrhizobium japonicum*. *Mol Plant Microbe Interact* 15 (12), 1228–1235.
- Luo, Q., Sun, L., Hu, X., Zhou, R., 2014. The variation of root exudates from the hyperaccumulator *Sedum alfredii* under cadmium stress: metabolomics analysis. *PLoS One* 9 (12), e115581.
- Sullivan, J.T., Trzebiatowski, J.R., Cruickshank, R.W., Gouzy, J., Brown, S.D., Elliot, R. M., et al., 2002. Comparative sequence analysis of the symbiosis island of *Mesorhizobium loti* strain R7A. *J Bacteriol* 184 (11), 3086–3095.

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Plant Growth Promoting Rhizobacteria: Application in Biofertilizers and Biocontrol of Phytopathogens

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3.1 INTRODUCTION

Climate change is the one of the most severe concerns for the government, policy makers as well as farmers from last two decades. The change in the climatic conditions is the ultimate results of global rising population. At the same time, there is a need to maintain food security for the rising global population through increases in crop production. Therefore farmers use huge amount of chemical fertilizers and pesticides to achieve maximum yields of crops due to limited land resources. These fertilizers are chemically synthesized, industrial substances composed of nitrogen, phosphorus, and potassium and their excess utilizations causes pollution to soil, air, and water directly or indirectly (Galloway et al., 2008; Youssef and Eissa, 2014). The continuous use of chemical fertilizers, biocides, and pesticides adversely affect the natural microflora such as bacteria, fungi, cyanobacteria, and protozoan present in the rhizosphere or the applied field and causes imbalance in the natural ecosystem (McLaughlin and Mineau, 1995; Dash et al., 2017a,b; Dash et al., 2018). Their long term application also severely health, texture, productivity of plants, and soil, which ultimately damage the environment as well as health and status of human well beings. In this context, sustainable agriculture is the need for the intricate problem of chemical fertilizers, pesticides, or ultimately for mitigation of climate changes (Kumar et al., 2017a).

Sustainable agriculture attracts the growing demand of biological-based organic fertilizers alternative to the agro-chemicals. Organic farming is a kind of agriculture, that not only ensures food security but also maintain the microbial diversity in soil. This types of farming influenced by natural micro flora of the soil, which constitutes plant growth promoting microorganisms such as bacteria, actinomycetes, arbuscular mycorrhiza fungi (AMF), cyanobacteria, which directly or indirectly associated with plants or soil for growth promotion, disease management, salt tolerance, drought tolerance, and also for the mitigation of heavy metal stress (Gupta et al., 2012; Govers et al., 2012; Kaushal and Wani, 2016).

The microbes present in the rhizosphere consist of various taxa such as bacteria, actinomycetes, fungi, protozoa, nematodes, and micro arthropods in decreasing order. The rhizosphere of the plant is a thin layer of soil, adhering to the root surface (Hiltner, 1904; Kumar et al., 2015b). In the rhizosphere of the plant, root secretes an array of exudates which contain large amount of carbohydrates, lipids, and amino acids, and these actively attract microbial population and colonize the plant roots, which directly or indirectly help in growth promotion and management of diseases or stress (Oku et al., 2012, Kumar et al., 2015a,b,c, 2016b, 2017a, b; Singh et al., 2017a,b,c).

Plant associated microbes affect the growth, yields, of host plant and also provide physiological and environmental advantage to the plants by various means. These microbes directly or indirectly involved in growth promotion, yield enhancement, hence term used for them is plant growth promoting microorganism, and the bacterial species associated with plant growth is called plant growth promoting bacteria (PGPB) (Kloepper et al., 2004; Glick et al., 2009; Babalola, 2010; Kumar et al., 2016b, 2017a).



3.2 PLANT GROWTH PROMOTING BACTERIA AS BIOFERTILIZER

Biofertilizers are artificially maintained cultures of the soil microorganism that can be used as microbial or soil inoculants to improve fertility and productivity of plant and soil. In another words, biofertilizer or microbial fertilizer is a substance composed of living microorganisms and mixture of biodegradable substances applied to seed, plant surfaces, or

soil, which colonizes the interior part of the plant, via various means such as rhizosphere, intercellular spaces, and enhanced the growth and yields by increasing availability of primary nutrients to the host plant (Vessey, 2003; Mazid et al., 2011). It is also considered as key factors to develop an integrated nutrient management system with very low environmental impact (Malusà et al., 2016).

The commercial history of biofertilizer start with the introduction of “Nitragin” by Nobbe and Hiltner, a laboratory culture of *Rhizobia* in 1895, followed by discovery of *Azotobacter* and then blue green algae (Ghosh, 2004; Mazid et al., 2011; Mazid and Khan, 2015; Das et al., 2015).

The beneficial plant–microbiome interactions represent a promising sustainable solution to improve agricultural production instead of chemical fertilizers (Timmusk et al., 2017). Biopesticides and biofertilizers are a part of natural-based products being widely use to enrich the quality of the soil and as biocontrol agent (Miranda, 2012). Currently different group of microorganisms have been identified, which belongs to bacteria, fungi, and protozoan kingdoms, these colonize rhizosphere or the internal plant tissues and used as biofertilizers for the enhanced agriculture production (Fig. 3.1) (Lucy et al., 2004; Smith and David, 2008; Vessey, 2003).

PGPB as biofertilizer has been proven as a safe and efficient methods of increasing crop yields (Premachandra et al., 2016; Vejan et al., 2016). Recently from last few decades numerous bacterial genera such as *Azotobacter*, *Bacillus*, *Klebsiella*, *Enterobacter*, *Arthrobacter*, *Burkholderia*, *Bacillus*, *Pseudomonas*, *Azotobacter Serratia*, etc. had been used as biofertilizers as reported by various authors and called these isolates as PGPB (Kloepper et al., 2004; Saharan and Nehra, 2011; Kumar et al., 2014, 2015a, 2016a,b, 2017a,b; Singh et al., 2017a).



3.3 MECHANISM OF ACTION

PGPBs as soil and plant inoculants broadly used in immanent decades across the world, and play an important role in improving nutrient and yield status of agricultural ecosystems by reducing the dependency of chemical fertilizers and pesticides (Zandi and Basu, 2016).

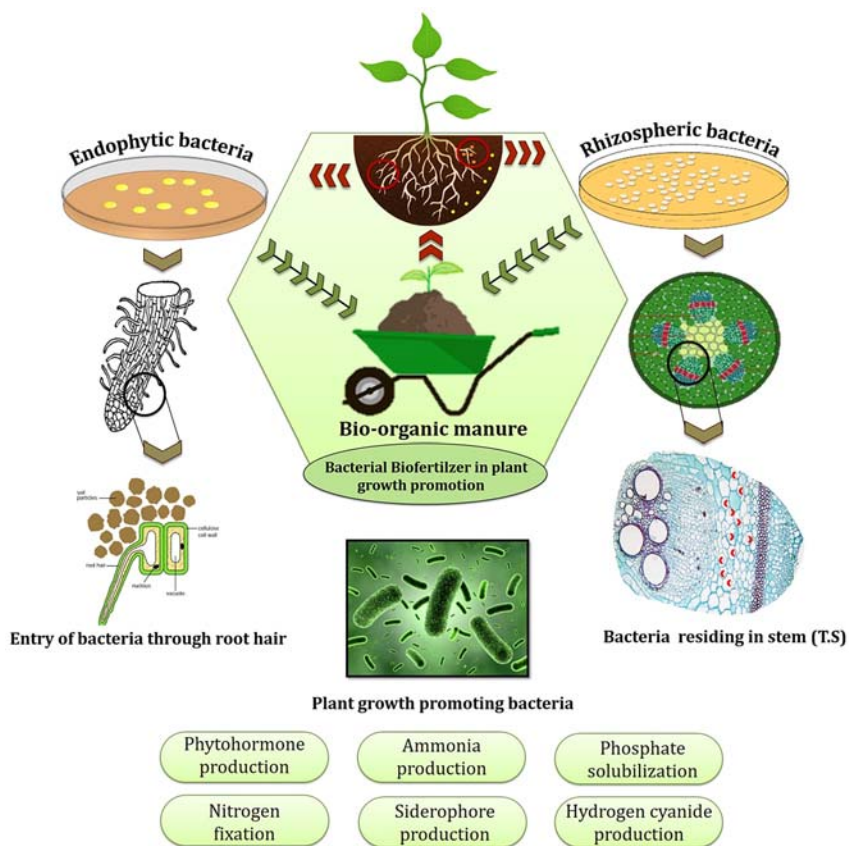


Figure 3.1 A schematic view of plant growth promoting bacteria (rhizospheric and endophytic) and their role in plant growth promotion.

PGPB promotes the growth of plant by either of direct and indirect mechanisms (Glick 1995). Direct mechanisms involve various processes such as phosphate solubilization, nitrogen fixation, production of siderophore, HCN, ammonia, vitamins, and phytohormones (such as auxin, cytokinin, and gibberellins) whereas indirect mechanisms involve that mechanism, which does not directly involve in growth promotion but plays role in path of synthesis. Indirect mechanisms include ACC deaminase activity, production of antibiotics, cell wall degrading enzymes, induced systemic resistance (ISR), etc. (Fig. 3.2) (Glick et al., 1999, Balogh et al., 2010; Frampton et al., 2012; Choudhary et al., 2011; Vejan et al., 2016, Kumar et al., 2016a,b, 2017a; Singh et al., 2017b,c).

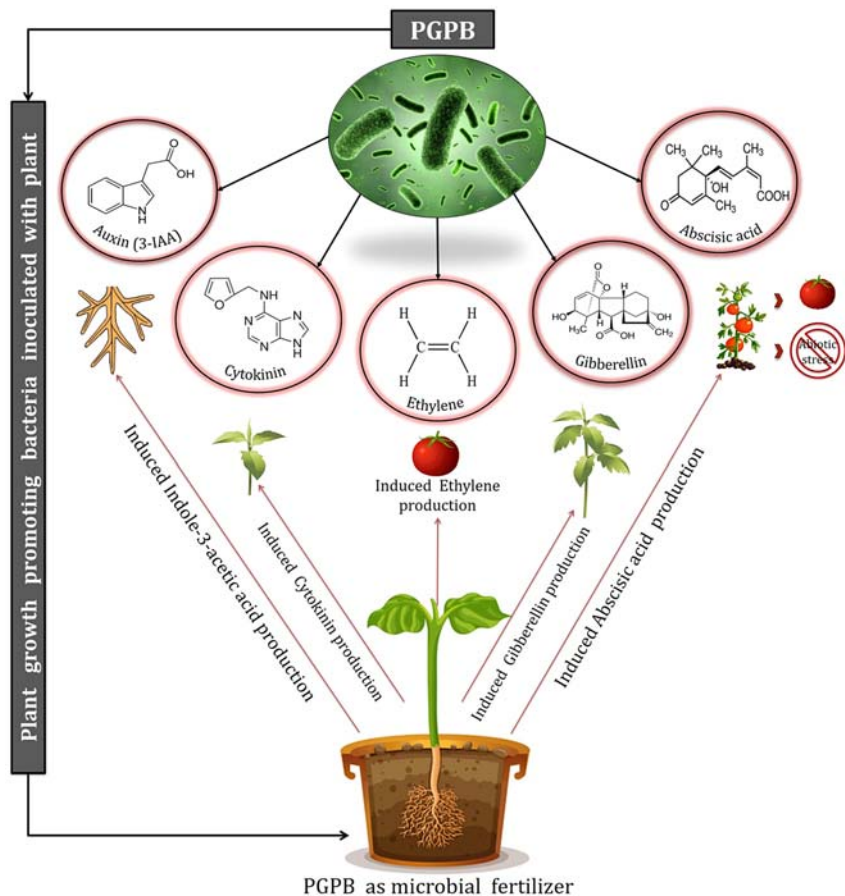


Figure 3.2 Role of biofertilizer to enhance the production of phytohormone and their impact on plant development and abiotic stress.

3.3.1 Phosphate solubilization

Phosphorus is the second most important nutrient for growth and development of plants. In nature, maximum amount of phosphorous present in the insoluble form of mineral salts, rock phosphate, tricalcium, dicalcium phosphate, hydroxyapatite, or organic compounds. Low availability of inorganic phosphate (orthophosphate) in soil seriously restricts the crop production (Hani, 2012; Miller et al., 2010; Wang et al., 2017). In this aspect, phosphate solubilizing bacteria (rhizosphere colonizing bacteria and endophytic) play an important role in liberating organic phosphates or to solubilize insoluble inorganic phosphate (Oteino et al., 2015).

In the previous study, many researchers reported role of phosphate solubilizing bacteria in plant growth promotion. Joe et al. (2016) used

Acinetobacter sp. and *Bacillus* sp., isolates of *Phyllanthus amarus* having salt tolerant and phosphate solubilizing property to reduce the consumption of fertilizer. They found inoculation of bacterial strains promoted higher vigor index, phosphorus content, percentage of germination, plant biomass, phenolic content, and also antioxidative activity compared to uninoculated control. In another study, Inagaki et al. (2015) used several PGPB strains as inoculants in acidic sandy soil, which showed higher concentration of phosphorus in the leaf tissue of maize.

3.3.2 Siderophore production

Siderophores are low-molecular-weight chelating agents (200–2000 Da) produced by bacteria, fungi, and plants to facilitate uptake of iron (Chu et al., 2010; Hider and Kong, 2010; Goswami et al., 2016). Iron is one of the most essential elements required for the development and normal functioning of plant and microorganisms. Although iron is abundantly available in the soil, but most of them is unavailable to the plant or other organisms, due to their presence in complex insoluble forms. Siderophores produced by PGPB helps in the fulfillment of required iron for plants by making it soluble and chelating from available complex organic or inorganic iron (Wandersman and Delepelaire, 2004; Arora et al., 2013; Singh et al., 2017a,b). Siderophores have a variety of chemical structures, which possess electron rich atoms such as oxygen or nitrogen electron donor atoms that can bind with metal cations (Chu et al., 2010; Hider and Kong, 2010; Verma et al., 2011; Ghavami et al., 2017).

Recently researches also find some another characteristics of siderophores besides the iron mobilization. In a study *Azotobacter vinelandii* nitrogen fixing bacteria produced siderophores that were also used in the acquisition of nitrogenase co-factors, molybdenum (Mo) and vanadium (V). An extensive study of siderophore production in *A. vinelandii* had been studied under presence of variety of trace metal. This showed the production of siderophores increases under Fe-limitation, while under Mo limitation only catechol type siderophore production has been increased (McRose et al., 2017). Many researchers reported siderophore producing bacterial strains, having prominent role in growth promotion and biocontrol activity (Kumar et al., 2016a, 2017b, Bindu and Nagendra, 2016). In a study, Venkat et al. (2017) reported *Bacillus* sp. and *Enterobacter* sp. isolates of iron-enriched soil had promising candidates for siderophores production and also potential role in medicinal and industrial

purposes. Yu et al. (2011) reported siderophore producing *Bacillus subtilis* isolates of pepper rhizosphere have bio-control activity against *Fusarium wilt* as well as growth promoting activity. The *Pseudomonas aeruginosa* isolated from rhizospheric soil also showed plant growth promotion as well as bio-control activity against chilli disease (Akhtar and Siddiqui, 2008; Sasirekha and Srividya, 2016). Similar types of observation were also reported by Bindu and Nagendra (2016) in case of paddy fields inoculated with *P. aeruginosa*.

3.3.3 Phytohormones production

Phytohormones regulate multiple aspects of growth promotion via various physiological and biochemical means to ensure a successful life cycle of plants (Waadt et al., 2015; Wani et al., 2016). These phytohormones prominently affect metabolic activity of plants for their normal functioning and also indirectly involved in stimulation of defense as well as abiotic stresses management. Abiotic stresses such as drought, salinity, heat, cold, flooding, and ultraviolet radiation are the severe problem resulting huge loss of crops production worldwide. In the mitigation of biotic and abiotic stresses, PGPB secretes various phytohormones or modulates the concentration of specific hormones in the plant. Phytohormone includes auxin, cytokinin, ethylene, gibberellins, and abscisic acid (ABA), where as some newer members of phytohormones have been also included in the list such as brassinosteroids, jasmonates, and strigolactones, which may appear as important metabolic engineering targets for producing abiotic stress-tolerant crop plants (Egamberdieva et al., 2017; Abd_Allah et al., 2018).

Indole-3-acetic acid (IAA), the most common whereas auxin is the most physiologically active phytohormone produced by PGPB, which directly involves in cell differentiation, cell division, and elongation of plants (Bhardwaj et al., 2014). Most of the PGPB secretes significant amount of IAA, which directly help in plant growth promotion (Kumar et al., 2015a). The concentration of IAA varies with the presence of different strains. Generally *Pseudomonas sp.* are the most potent producer of IAA in comparison to other bacterial genera, where as *Pseudomonas putida* is superior to *P. fluorescens* in production of IAA (Bharucha et al., 2013; Reetha et al., 2014; Kumar et al., 2015a).

Ethylene the first gaseous phytohormone, and have pronounced effects on growth and development of plants. It is one of the simplest phytohormone can function even at very low concentrations (Abeles et al., 1992).

The concentration of ethylene controls the growth and senescence of plant (Reid, 1995; Lutts et al., 1996; Thompson et al., 1998; Pierik et al., 2006; Masood et al., 2012; Nazar et al., 2014). In case of higher plants, under normal or in stress conditions, ethylene is produced from S-adenosyl-Methionine via the action of enzyme ACC synthetase (Abeles et al., 1992). Presence of 1-aminocyclopropane-1-carboxylate (ACC) deaminase producing bacteria, either in rhizosphere or endophytes, plays an active role in modulation of ethylene levels in the plants (Gamalero and Glick, 2015). In a study, it has been found that inoculation of *petunia* explants with *A. rhizogenes* A4 and LBA strains increased the ethylene production in the explants (KVpczynska et al., 2003).

Absciscic acids (ABAs) are key hormones involved in tuning responses against several environmental stresses and have remarkable impact on the plant defense against various pathogens (Alazem and Lin, 2017; Davies and Zhang, 1991). ABA enhanced plant defense mechanism, if triggered at early stages of infection or by closing stomata or by inducing callose deposition in the cell walls. There is very few report of ABA production by bacteria present. Tuomi and Rosenquist (1995) determined ABA, IAA, and GA3 production and their impact in *Bacillus amyloliquefaciens* inoculated *Oriza sativa*, after inoculation. They found significant increase in growth attributes of rice plants as compared to noninoculated control plants under salinity stress (Shahzad et al., 2017). In another study, *P. putida* used in ameliorating drought stress by the production of ABA, after inoculation with chickpea cultivars (Tiwari et al., 2016). *Bacillus licheniformis* Rt4M10 and *Pseudomonas fluorescens* Rt6M10 isolated from rhizospheric region and at different depths of rhizospheric soil of grapevines, both the strains produced ABA, indole- 3-acetic acid (IAA), and the gibberellins. The concentration of ABA increased as compared to control in 45 days-old *Vitis vinifera* plants after inoculation with *B. licheniformis* and *P. fluorescens*, respectively (Salomon et al., 2014). *Bacillus licheniformis* SA03 inoculated with *Chrysanthemum* plants grown under saline-alkaline conditions, significantly decreased saline-alkaline stress in plants along with augmented photosynthesis, biomass and survival rates via mediating cellular ABA levels (Zhou et al., 2017).

3.3.4 Ammonia and hydrogen cyanide production

The production of hydrogen cyanide and ammonia (NH₃) are important PGP activity of plant growth promoting strains. HCN commonly used as

a biocontrol agent in the agriculture system on the basis of significant toxicity against phytopathogens on the other hand HCN also used in the chelating of metals ions as well as indirectly involved in making availability of phosphate (Rijavec and Lapanje, 2016). The production and synthesis of HCN by PGPR (Plant growth promoting rhizobacteria) are independent of their genus, and their impact suggested their possibility to use as biological fertilizers or biocontrol to enhance crop production (Agbodjato et al., 2015; Rijavec and Lapanje, 2016). Many authors reported HCN producing PGPR and their use as a biofertilizer in growth promotion and yield enhancement (Rijavec and Lapanje, 2016; Kumar et al., 2016a,b). In a study, Heydari et al. (2008) isolated cyanogenic strain of *Pseudomonas fluorescense* and reported potential role as biocontrol, enhancement in length of stem and roots, germination rate of rye, wild barley, and wheat. Besides HCN, ammonia production by PGPR accumulates and supplies nitrogen to their host plant and promotes root and shoot elongation and their biomass (Marques et al., 2010). Some of the PGPR strains have potential to synthesize HCN as well as ammonia and their synergetic effect on plant growth as well as modulation of plant metabolites Agbodjato et al., 2015; Kumar et al., 2016b).

3.3.5 Enzyme production

Plant contains different types of enzymes that help in regulation of different metabolic activity of plants. These include catalases, ascorbate peroxidases (APX), peroxiredoxins (PRX), glutathione/thioredoxin peroxidases (GPX), and glutathione S-transferases (GST) (Willekens et al., 1995; Asada, 1999; Wagner et al., 2002; Dietz, 2003; Mittler et al., 2004; Iqbal et al., 2006).

Some of the enzyme such as (H_2O_2) act as signal molecule that involved in regulation of biological/physiological processes such as photosynthetic functions, cell cycle, and plant responses to withstand biotic and abiotic stresses (Sofo et al., 2015). In another study, several PGPR strains such as *Pseudomonas*, *Bacillus*, *Xanthomonas*, and *Agrobacterium sp.* isolated from *Valeriana officinalis* showed strong production protease and lipase enzymes (Ghodsolavi et al., 2013).

The enhanced gene expression of various antioxidant enzymes such as ascorbate peroxidase (APX), manganese-dependent superoxide dismutase (MnSOD), catalase (CAT), peroxidase (POD), glutathione peroxidase (GPX) and glutathione reductase (GR), and higher proline content in

PGPR inoculated wheat plants contributed to increased tolerance to salinity stress (Bharti et al., 2016). In another study, rice plants inoculated with PGPR strains, *Pseudomonas* sp. Rh323 and *Pseudomonas* sp. showed strong polyphenol oxidase (PPO) activity in leaves of rice plants, while peroxidase (PO) and phenylalanine ammonia-lyase (PAL) activities were maximum in plants inoculated with *Pseudomonas* sp. as compared to control plant (Yasmin et al., 2016).

3.3.6 Nitrogen fixation

Nitrogen is the seventh most abundant element in the universe. It's the most common element in the atmosphere comprising about 78% of the gas. It is present in the soil abundantly but unavailable to the plants due to insoluble forms. Plants uptake nitrogen in the form of ammonium (NH_4^+) and nitrate (NO_3^-). Conversion of atmospheric N_2 to ammonium is known as nitrogen fixation or diazotrophy. The ability to fix nitrogen is widespread among prokaryotes with representatives in both bacteria and archaea (Dekas et al., 2009; Das et al., 2015).

PGPR may provide a biological alternative to fix atmospheric N_2 and delay N remobilization as it is directly correlated to plant senescence (Kuan et al., 2016). In a study, rice plants inoculated with endophytic strains showed a significant up regulation of the nitrogenase activity. The overproduction of IAA in endophytes up regulates nitrogen fixation in both bacterial cultures and inoculated rice plants. These strains could be used as biofertilizer to improve the growth and the yield of agricultural crops, offering an alternative to the use of chemical nitrogen fertilizers (Defez et al., 2017).



3.4 MICROBIAL INOCULATION FOR THE PLANT GROWTH PROMOTION

Bacterial inoculants as plant or soil inoculants can contribute to increase agronomic efficiency by reducing production costs and environmental pollution. A large number of bacteria including species of *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthobacter*, *Burkholderia*, *Bacillus*, and *Serratia* have been reported to enhance plant growth after inoculation with PGPB strains (Table 3.1, Fig. 3.3)

Table 3.1 Plant growth promoting bacteria (PGPB) as bio-fertilizer and their plant growth promotion (PGP) activity

S.N	PGPB strains	Region	PGP activity	Plant	Reference
1	<i>Alcaligenes faecalis</i> sub sp. <i>faecalis</i> str. S8	Endophyte	P. solubilization IAA	<i>Withania somnifera</i>	Abdallah et al. (2016)
2	<i>Achromobacter xylosoxidans</i> Fd2 <i>Herbaspirillum seropedicae</i> Oci9, <i>Ochrobactrum rhizosphaerae</i> Oci13	Rhizosphere	IAA, Siderophore	<i>Ocimum sanctum</i>	Barnawal et al. (2012)
3	<i>Serratia Ureilytica</i> Bac5	Rhizosphere	Siderophore, ACC deaminase P. solubilization	<i>Ocimum sanctum</i>	Barnawal et al. (2012)
4	<i>Pseudomonas stutzeri</i> CSP03 <i>Bacillus subtilis</i> TTP02, <i>Pseudomonas putida</i> PHP03	Rhizosphere	IAA, N ₂ fixation, P. solubilization Siderophore	<i>Capparis spinosa</i>	El-Sayed et al. (2014)
5	<i>Enterobacter</i> sp. TAP02	Rhizosphere	IAA, N ₂ fixation, P. solubilization	<i>T. amplexicaulis</i>	El-Sayed et al. (2014)
6	<i>Bacillus</i> sp. <i>Pseudomonas putida</i> (ECL5)	Endophyte	IAA, P. solubilization Siderophore	<i>Curcuma longa</i> L.	Kumar et al. (2016a,b)
7	<i>Clavibacter michiganensis</i>	Endophyte	IAA,	<i>Curcuma longa</i> L.	Kumar et al. (2016a,b)
8	<i>Azotobacter chroococcum</i> CL13	Rhizosphere	IAA, HCN, ammonia production, phosphate solubilization	<i>Curcuma longa</i> L.	Kumar et al. (2014)
9	<i>Achromobacter xylosoxidans</i> AUM54	Endophyte	IAA, P. solubilization Siderophore	<i>Catharanthus roseus</i>	Karthikeyan et al. (2012)
10	<i>Bacillus subtilis</i> LK14	Endophyte	P. solubilization IAA,	<i>Moringa peregrina</i>	Khan et al. (2016)

(Continued)

Table 3.1 (Continued)

S.N	PGPB strains	Region	PGP activity	Plant	Reference
11	<i>B. subtilis</i> CT-1 <i>A. tumefaciens</i> CT-2 <i>Bacillus</i> sp., CT-3 <i>P. putida</i> CT-4, <i>Pseudomonas</i> sp., CT-5	Endophyte	IAA, Ammonia, P. solubilization	<i>Cassia tora</i> L.	Kumar et al. (2015a)
12	<i>Pseudomonas stutzeri</i> P3	Endophyte	IAA production	<i>Echinacea</i>	Lata et al. (2006)
13	<i>Brevundimonas diminuta</i> EGE-B-1 <i>Agrobacterium tumefaciens</i> EGE-B-5 <i>Stenotrophomonas rhizophila</i> EGE-B-6	Endophyte	IAA, P. solubilization	Peach	Liaqat and Eltem (2016)
14	<i>Stenotrophomonas maltophilia</i> R551-3	Endophyte	IAA synthesis, ACC deaminase	Poplar	Taghavi et al. (2009)
15	<i>Paenibacillus durus</i> BR 30	Rhizosphere	IAA, N ₂ fixation, P. solubilization	<i>Asphodelus</i> sp.	Navarro-Noyaa et al. (2012)
16	<i>Paenibacillus borealis</i> BR 32	Rhizosphere	IAA, N ₂ fixation, P. solubilization	<i>Juniperus</i> sp.	Navarro-Noyaa et al. (2012)
17	<i>Paenibacillus graminis</i> BR 35	Rhizosphere	N ₂ fixation, P. solubilization	<i>Aster gymnocephalus</i>	Navarro-Noyaa et al. (2012)
18	<i>Azospirillum lipoferum</i> KYR F6	Rhizosphere	IAA, N ₂ fixation, P. solubilization	<i>Haplopappus</i> sp.	Navarro-Noyaa et al. (2012)
19	<i>Arthrobacter</i> sp. SMR3, <i>B. subtilis</i> SMR15	Endophyte	IAA, ACC deaminase	<i>Papaver somniferum</i>	Pandey et al. (2016)
20	<i>Pseudomonas</i> BA-8, <i>Bacillus</i> OSU-142, <i>Bacillus</i> M-3	Rhizosphere	Auxin and Cytokinins	Strawberry	Pırlak and Kose (2009) Aslantas et al. (2007)
21	<i>Rhizobium</i> sp. <i>Azospirillum</i> sp.	Endophyte	IAA, N ₂ fixation	<i>Oryza sativa</i>	Sev et al. (2016)
22	<i>Rhizobium</i> sp.,	Rhizosphere	Cytokinin production	<i>Mimosa pudica</i>	Sabat et al. (2014)

23	<i>Pseudomonas aeruginosa</i> FTR, <i>Enterobacter asburiae</i> MRC12 <i>Acinetobacter brumalii</i> MZ30V92	Endophyte	Ammonia, P. solubilization Siderophore, HCN	Maize	Sandhya et al. (2017)
24	<i>Pseudomonas monteilii</i> FMZR2 <i>Sinorhizobium meliloti</i> MRC31	Endophyte	Ammonia, P. solubilization, HCN	Maize	Sandhya et al. (2017)
25	<i>Acinetobacter sp.</i> ALEB16	Endophyte	abscisic acid (ABA), salicylic acid (SA)	<i>Atractylodes lancea</i>	Wang et al. (2015)
26	<i>Serratia sp.</i> Rh269	Rhizosphere	IAA, Siderophore P. solubilization	Rice	Yasmin et al. (2016)
27	<i>Bacillus sp.</i> Rh219	Rhizosphere	Siderophore	Rice	Yasmin et al. (2016)
28	<i>Pseudomonas sp.</i> E227	Rhizosphere	IAA, Siderophore, HCN, P. solubilization	Rice	Yasmin et al. (2016)

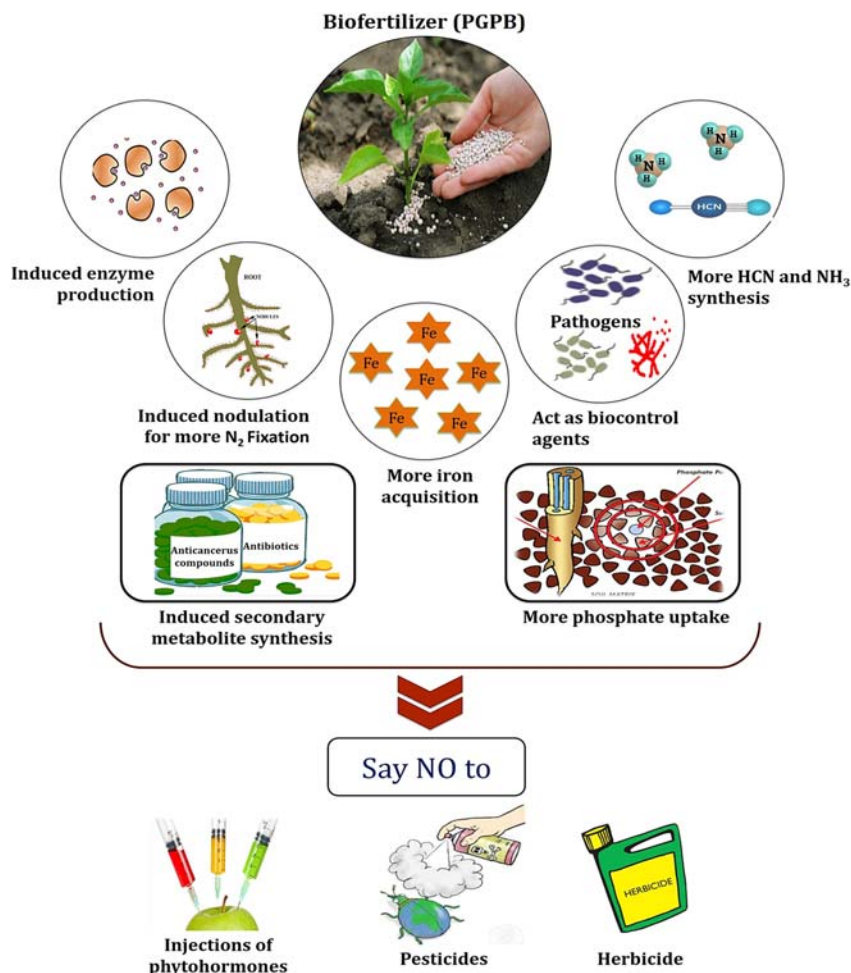


Figure 3.3 Overview of microbial inoculation on the growth and disease management in plant.

(Glick, 1995; Kloepper et al., 1989; Okon and Labandera-Gonzalez, 1994; Chelius and Triplett, 2001; Montañez et al., 2009; Piromyou et al., 2011; Zakry et al., 2012; Arruda et al., 2013; Souza et al., 2015.)

Plant growth-promoting bacteria (PGPB) that produce 1-aminocyclopropane-1-carboxylate deaminase (ACC deaminase) in the presence of abiotic stresses by reducing stress ethylene, inoculated with *Camelina sativa* facilitate plant growth, seed production, and seed quality that is not suitable for the majority of crops due to high salt content (Hontzeasa et al., 2004; Heydarian et al., 2016). Currently many reports

are present related with PGPB impact on the growth modulation and yield enhancement. Khan et al. (2016) reported PGP activity of *B. subtilis* after inoculation in *Solanum lycopersicum*, which significantly increased the shoot biomass, root biomass, and chlorophyll (a and b) contents as compared to control plants. Cura et al. (2017) used *Azospirillum brasilense* and *Herbaspirillum seropedicae* for the inoculation in corn (*Zea mays* L.), which showed higher tolerance to water stress, drought conditions, along with high biomass production; enhanced carbon, nitrogen, and chlorophyll levels and also lower the levels of ABA and ethylene. In another study *Bacillus* sp., *Oceanobacillus oncorhynch*, *Exiguobacterium aurantiacum* inoculated with wheat, which showed significant increase in the length of root and shoot, and total fresh weight of plant after inoculations (Orhan, 2016). In another study, Inagaki et al. (2015) reported seeds inoculated with bacterial consortium (*A. brasiliense* AbV5 + *H. seropedicae* SMR1) improved leaf area, stem diameter, relative chlorophyll content, while seed inoculation with *H. seropedicae* enhanced the concentration of nitrogen in the leaf tissue of maize under acidic conditions without supply of nitrogen in the soil.

Kuan et al. (2016) reported *Klebsiella* sp., *Klebsiella pneumonia*, *Bacillus pumilus* *Acinetobacter* sp. and *B. subtilis* from the roots of maize, all the strains were positive for nitrogen fixation, phosphate solubilization, and IAA production and significantly used in plant growth promotion as bio-fertilizer. *Bacillus* sp. isolated from *Phaseolus vulgaris* L., produced IAA, siderophore, phytase, organic acid, ACC deaminase, cyanogens, lytic enzymes, oxalate oxidase, and solubilized various sources of organic, inorganic phosphates. This strain also inhibited the growth of several phytopathogens such as *Macrophomina phaseolina*, *Fusarium oxysporum*, *F. solani*, *Sclerotinia sclerotiorum*, *Rhizoctonia solani*, and *Colletotricum* sp. (Kumar et al., 2012). In another study, *Bacillus* OSU-142 and *Bacillus* M-3 in sugar beet and barley (Cakmakci et al., 2001), tomatoes (Turan et al., 2004), and apple (Aslantas et al., 2007), had been also reported by respective authors in respect to enhanced yield and quality of respected plants. In addition, floral and foliar application of *Bacillus* OSU-142 increased yield and growth in apricot (Esitken et al., 2002, 2003).



3.5 PLANT GROWTH PROMOTING BACTERIA AS BIOCONTROL

The developments of diseases in plants are the predominant factor in reducing crop yield, declining production quality, and contamination of food grains. The incessant variety and intricacy of plant diseases have led to the development of pesticides (Guo et al., 2013; Dun-chun et al., 2016). Unfortunately, the continuous use of these pesticides has developed resistance in phytopathogens and gives rise to several environmental concerns. Biological control is contemplated as an alternative of pesticides to control phytopathogen (Compant et al., 2005). The use of PGPB as a biological agent stimulates plant growth and manages plant health. PGPB has several advantages over conventional pest control method. The application of PGPB in the agricultural field is sustainable and non toxic. PGPB can limit or prevent damage caused by phytopathogens via different mechanisms (Olanrewaju et al., 2017).

Antibiotics production is the main mechanism of PGPB to counteract the damaging effects of phytopathogens. A variety of compounds such as 2,4-diacetylphloroglucinol (DAPG), amphisin, hydrogen cyanide, phenazine, oomycin A, tropolone, pyoluteorin, tensin, pyrrolnitrin, and cyclic lipopeptides produced by *Pseudomonads*, and kanosamine, oligomycin A, xanthobaccin and zwittermicin produced by *Streptomyces*, *Bacillus*, and *Stenotrophomonas spp.* have been identified as antibiotics that have antibacterial, antifungal, antiviral, antihelminthic, antimicrobial, cytotoxic, phytotoxic, antioxidant, and antitumor properties.

Several bacteria suppress phytopathogen activity by producing enzymes to hydrolyze the cellulose, hemicelluloses, chitin, and proteins. The chitinase produced by *Serratia plymuthica* C48, *Serratia marcescens*, *Paenibacillus sp.*, *Streptomyces sp.* and *Pseudomonas stutzeri* degrade mycelia of various fungal phytopathogens. The β -1,3-glucanase synthesized by *Streptomyces*, *Paenibacillus*, and *Bacillus sp.* lyses fungal cell wall. Similarly, protease and lipase produced by PGPB could degrade proteins and lipids associated to cell wall. *Pseudomonas*, *Rhizobium*, *Bacillus*, *Alcaligenes*, and *Aeromonas* produce hydrogen cyanide that enhances the effectiveness of antifungal activity of these bacteria (Compant et al., 2005; Guo et al., 2013; Olanrewaju et al., 2017).

In addition, siderophore production is one of the effective mechanisms of PGPB to impede propagation of phytopathogens. These siderophores act as iron-chelators, and bind most of the iron presents in rhizospheric region.

Thus, the unavailability of iron in rhizosphere region prevents proliferation of bacterial and fungal pathogens (Olanrewaju et al., 2017).

PGPB may also induce systemic resistance against phytopathogens. The ISR is a key mechanism to enhance plant defense against various pathogens by *Pseudomonas*, *Trichoderma*, *Bacillus*, and mycorrhiza. ISR is the consequence for specific recognition of pathogen by plant receptors (Pieterse et al., 2014). Several PGPBs produce salicylic acid that stimulates systemic acquired resistance (SAR), a mechanism similar to ISR.

Detoxification of phytopathogen virulence factor is another effective mechanism of biocontrol. Certain PGPB can detoxify toxin produced by *Xanthomonas albilineans* and *Fusarium* species (Compant et al., 2005). Many PGPB used quorum sensing as mechanism to regulate production of virulence factor. They checked the quorum sensing capacity of pathogens by impairing autoinducer signals, and thus arresting the expression of virulence factor (Olanrewaju et al., 2017).

Recently, the use of bacteriophage as biocontrol agent is a promising but unusual practice. Phages have natural potential to overcome the problem of phage-resistance or new strains of bacteria. They can be used with other biocontrol agents. A restriction to their application is sensitivity toward UV light, thus to conquer this problem they are sprayed on the plant at evening (Buttimer et al., 2017). Besides applying as biocontrol agent, they can be used in phage-based diagnostics of phytopathogenic bacteria.

In recent years, sustainable agriculture becomes the requirement for the world because of undesirable effects of the chemical pesticides on the environment as well as on the human beings. The use of PGPB to improve crop production has been demonstrated in various studies, which reveal the opportunities to improve crop nutrition, yield and disease management (Kumar et al., 2015c; Singh et al., 2017b,c). The use of PGPB has reduced the chemical inputs in soil, and manages the environmental pollution. PGPB is one among the suitable alternatives that have been executed as growth promoting and biocontrol agents (Kumar et al., 2017a,b; Singh et al., 2017a,b,c).



3.6 CONCLUSION

Generally chemical fertilizers and pesticides are effective and convenient in use for production and disease management of plants but they are

potential threat for the health and environment of soil, plant as well as humans. Therefore, use of PGPB as biofertilizer and biocontrol agents is the sustainable method for agriculture due to their less side effect and profitable agricultural productivity. Besides biofertilizers and biocontrol, microbial inoculants or PGPB currently also used in the salinity, draught, and other biotic and abiotic stress management. It opens a new door for the farmers, industries, and researchers to use these PGPB strains in the tolerance of salinity, heavy metal and also in xenobiotic degradation? These PGPB strains currently also used in bioactive compounds production, which mediate in the drug discovery and helps in disease management.

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REFERENCES

- Abd_Allah, E.F., Alqarawi, A.A., Hashem, A., Radhakrishnan, R., Al-Huqail, A.A., Al-Otibi, F.O.N., et al., 2018. Endophytic bacterium *Bacillus subtilis* (BERA 71) improves salt tolerance in chickpea plants by regulating the plant defense mechanisms. *J Plant Interact* 13 (1), 37–44.
- Abdallah, R.A.B., Trabelsi, B.M., Nefzi, A., Khiareddine, H.J., Remadi, M.D., 2016. Isolation of Endophytic bacteria from *Withania somnifera* and assessment of their ability to suppress Fusarium Wilt disease in tomato and to promote plant growth. *J Plant Pathol Microbiol* 7, 352.
- Abeles, F.B., Morgan, P.W., Saltveit Jr., M.E., 1992. Ethylene in Plant Biology, second ed Academic Press, New York.
- Agbodjato, N.A., Noumavo, P.A., Baba-Moussa, F., Salami, H.A., Sina, H., Sèzan, A., et al., 2015. Characterization of potential plant growth promoting rhizobacteria isolated from maize (*Zea mays* L.) in central and northern Benin (West Africa). *Appl Environ Soil Sci* 2015, 1–11.
- Akhtar, M.S., Siddiqui, Z.A., 2008. Arbuscular mycorrhizal fungi as potential bioprotectants against plant pathogens. In: Siddiqui, Z.A., Akhtar, M.S., Futai, K. (Eds.), *Mycorrhizae: Sustainable Agriculture and Forestry*. Springer, Dordrecht.
- Alazem, M., Lin, N.S., 2017. Antiviral roles of abscisic acid in plants. *Front Plant Sci* 8, 1760.
- Arora, N.K., Tewari, S., Singh, R., 2013. Multifaceted plant-associated microbes and their mechanisms diminish the concept of direct and indirect PGPRs. In: Arora, N. (Ed.), *Plant Microbe Symbiosis: Fundamentals and Advances*. Springer, New Delhi.
- Arruda, L., Beneduzi, A., Martins, A., Lisboa, B., Lopes, C., Bertolo, F., et al., 2013. Screening of rhizobacteria from maize (*Zea mays* L.) in Rio Grande do Sul State (South Brazil) and analysis of their potential to improve plant growth. *Appl Soil Ecol* 63, 15–22.
- Asada, K., 1999. The water-water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. *Annu Rev Plant Biol* 50 (1), 601–639.

- Aslantas, R., Cakmakci, R., Sahin, F., 2007. Effect of plant growth promoting rhizobacteria on young apple tree growth and fruit yield under orchard conditions. *Sci Hortic* 111, 371–377.
- Babalola, O.O., 2010. Beneficial bacteria of agricultural importance. *Biotechnol Lett* 32 (11), 1559–1570.
- Balogh, B., Jones, J.B., Iriarte, F., Momol, M., 2010. Phage therapy for plant disease control. *Curr Pharm Biotechnol* 11, 48–57.
- Barnawal, D., Bharti, N., Maji, D., Chanotiya, C.S. and Kalra, A., 2012. 1-Aminocyclopropane-1 carboxylic acid (ACC) deaminase-containing rhizobacteria protect *Ocimum sanctum* plants during waterlogging stress via reduced ethylene. *Plant Physiol Biochem* 58, 227–235.
- Bhardwaj, D., Ansari, M.W., Sahoo, R.K., Tuteja, N., 2014. Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity. *Microb Cell Fact* 13 (1), 66.
- Bharti, N., Pandey, S.S., Barnawal, D., Patel, V.K., Kalra, A., 2016. Plant growth promoting rhizobacteria *Dietzia natronolimnaea* modulates the expression of stress responsive genes providing protection of wheat from salinity stress. *Sci Rep* 6, 34768.
- Bharucha, U., Kamlesh, P., Ujjval, B., Trivedi, 2013. Optimization of indole acetic acid production by *Pseudomonas putida* UB1 and its effect as plant growth-promoting rhizobacteria on mustard (*Brassica nigra*). *Agric Res* 2 (3), 215–221.
- Bindu, P., Nagendra, P.G., 2016. Siderophore production by *Pseudomonas aeruginosa* isolated from the paddy fields of Kuttanad, Kerala. *Int J Sci Res* 1577–1581.
- Buttimer, C., McAuliffe, O., Ross, R.P., Hill, C., O'Mahony, J., Coffey, A., 2017. Bacteriophages and bacterial plant diseases. *Front Microbiol* 8, 34.
- Cakmakci, R., Kantar, F., Sahin, F., 2001. Effect of N₂-fixing bacterial inoculations on yield of sugar beet and barley. *J Plant Nutr Soil Sci* 164, 527–531.
- Chelius, M.K., Triplett, E.W., 2001. The diversity of archaea and bacteria in association with the roots of *Zea mays* L. *Microb Ecol* 41, 252–263.
- Choudhary, D.K., Sharma, K.P., Gaur, R.K., 2011. Biotechnological perspectives of microbes in agro-ecosystems. *Biotechnol Lett* 33, 1905–1910.
- Chu, B.C., Garcia-Herrero, A., Johanson, T.H., Krewulak, K.D., Lau, C.K., Peacock, R.S., et al., 2010. Siderophore uptake in bacteria and the battle for iron with the host; a bird's eye view. *Biometals* 23 (4), 601–611.
- Compant, S., Reiter, B., Sessitsch, A., Nowak, J., Clément, C., Barka, E.A., 2005. Endophytic colonization of *Vitis vinifera* L. by plant growth-promoting bacterium *Burkholderia* sp. strain PsJN. *Appl Environ Microbiol* 71 (4), 1685–1693.
- Cura, J.A., Franz, D.R., Filosofia, J.E., Balestrasse, K.B., Burgueno, L.E., 2017. Inoculation with *Azospirillum* sp. and *Herbaspirillum* sp. bacteria increases the tolerance of maize to drought stress. *Microorganisms* 5, 41.
- Das, N.P., Kumar, A., Singh, P.K., 2015. Cyanobacteria, pesticides and rice interaction. *Biodivers Conserv* 24 (4), 995–1005.
- Dash, N.P., Kumar, A., Kaushik, M.S., Abraham, G., Singh, P.K., 2017a. Agrochemicals influencing nitrogenase, biomass of N₂-fixing cyanobacteria and yield of rice in wetland cultivation. *Biocatal Agric Biotechnol* 9, 28–34.
- Dash, N.P., Kumar, A., Kaushik, M.S., Abraham, G., Singh, P.K., 2017b. Nitrogenous agrochemicals inhibiting native diazotrophic cyanobacterial contribution in wetland rice ecosystem. *J Appl Microb* 29 (2), 929–939.
- Dash, N.P., Kaushik, M.S., Kumar, A., Abraham, G., Singh, P.K., 2018. Toxicity of biocides to native cyanobacteria at different rice crop stages in wetland paddy field. *J Appl Phycol* 30 (1), 483–493.
- Davies, W.J., Zhang, J., 1991. Root signals and the regulation of growth and development of plants in drying soil. *Annu Rev Plant Physiol Plant Mol Biol* 42, 55e 76.

- Defez, R., Anna Andreozzi, A., Bianco, C., 2017. The overproduction of Indole-3-acetic acid (IAA) in endophytes upregulates nitrogen fixation in both bacterial cultures and inoculated rice plants. *Microb Ecol* 74 (2), 441–452.
- Dekas, A.D., Poretsky, R.S., Orphan, V.J., 2009. Deep-sea archaea fix and share nitrogen in methane-consuming microbial consortia. *Science* 326, 422–426.
- Dietz, K.J., 2003. Plant peroxiredoxins. *Annu Rev Plant Biol* 54 (1), 93–107.
- Dun-chun, H.E., Jia-sui, Z.H.A.N., Lian-hui, X.I.E., 2016. Problems, challenges and future of plant disease management: from an ecological point of view". *J Integr Agric*, 15 4, 705–715.
- Egamberdieva, D., Wirth, S.J., Alqarawi, A.A., Abd_Allah, E.F., Hashem, A., 2017. Phytohormones and beneficial microbes: essential components for plants to balance stress and fitness. *Front Microbiol* 8, 2104.
- El-Sayed, W.S., Akhka, A., El-Naggar, M.Y., Elbadry, M., 2014. In vitro antagonistic activity, plant growth promoting traits and phylogenetic affiliation of rhizobacteria associated with wild plants grown in arid soil. *Front Microbiol* 5, 651.
- Esitken, A., Karlidag, H., Ercisli, S., Sahin, F., 2002. Effects of foliar application of *Bacillus subtilis* Osu-142 on the yield, growth and control of shot-hole disease (*Coryneum blight*) of apricot. *Gartenbauwissenschaft* 67, 139–142.
- Esitken, A., Karlidag, H., Ercisli, S., Turan, M., Sahin, F., 2003. The effect of spraying a growth promoting bacterium on the yield, growth and nutrient element composition of leaves of apricot (*Prunus armeniaca* L. cv. Hacihaliloglu). *Aust J Agric Res* 54, 377–380.
- Frampton, R.A., Pitman, A.R., Fineran, P.C., 2012. Advances in bacteriophage-mediated control of plant pathogens. *Int J Microbiol*, 2012, 326452.
- Galloway, J.N., Townsend, A.R., Erisman, J.W., Bekunda, M., Cai, Z., Freney, J.R., et al., 2008. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. *Science* 320 (5878), 889–892.
- Gamalero, E., Glick, B.R., 2015. Bacterial modulation of plant ethylene levels. *Plant Physiol* 169, 13–22.
- Ghavami, N., Alikhani, H.A., Pourbabaei, A.A., Besharati, H., 2017. Effects of two new siderophore-producing rhizobacteria on growth and iron content of maize and canola plants. *J Plant Nutr* 40 (5), 736–746.
- Ghodsolavi, B., Ahmadzadeh, M., Soleimani, M., Madloo, P.B., Taghizad-Farid, R., 2013. Isolation and characterization of rhizobacteria and their effects on root extracts of *Valeriana officinalis*. *Aust J Crop Sci* 7 (3), 338–344.
- Ghosh, N., 2004. Promoting biofertilisers in Indian agriculture. *Econ Polit Wkly* 5, 5617–5625.
- Glick, B.R., 1995. The enhancement of plant growth by free living bacteria. *Can J Microbiol* 41, 109–114.
- Glick, B.R., Patten, C.L., Holguin, G., Penrose, D.M., 1999. *Penrose Biochemical and Genetic Mechanism Used by Plant Growth Promoting Bacteria*. Imperial College Press, London, UK.
- Goswami, D., Thakker, J.N., Dhandhukia, P.C., Tejada, M.M., 2016. Portraying mechanisms of plant growth promoting rhizobacteria (PGPR): a review. *Cogent Food Agric* 2, 1127500.
- Govers, G., Merckx, R., Van Oost, K., van Wesemae, B., 2012. Soil organic carbon management for global benefits: A discussion paper. *Soil Organic Carbon Benefits: A Scoping Study*. G.G.E.F Scientific and Technical Advisory Panel, Nairobi, Kenya.
- Guo, R.F., Yuan, G.F., Wang, Q.M., 2013. Effect of NaCl treatments on glucosinolate metabolism in broccoli sprouts. *J Zhejiang Univ Sci B* 14 (2), 124.

- Gupta, G., Panwar, J., Akhtar, M.S., Jha, P.N., 2012. Endophytic nitrogen-fixing bacteria as biofertilizer. In: Sustainable Agriculture Reviews. Springer, Netherlands, pp. 183–221.
- Hani, A., 2012. Beneficial microorganisms for the sustainable use of phosphates in agriculture. *Procedia Eng* 46, 62–67.
- Heydari, S., Moghadam, P.R. and Kennedy Arab, S.M., 2008. Hydrogen Cyanide Production Ability by *Pseudomonas* Fluorescence Bacteria and their Inhibition Potential on Weed germination, In Proceedings “Competition for Resources in a Changing World: New Drive for Rural Development”, Tropentag, Hohenheim.
- Heydarian, Z., Yu, M., Gruber, M., Glick, B.R., Zhou, R., Hegedus, D.D., 2016. Inoculation of soil with plant growth promoting bacteria producing 1-Aminocyclopropane-1-Carboxylate deaminase or expression of the corresponding *acdS* gene in transgenic plants increases salinity Tolerance in *Camelina sativa*. *Front Microbiol* 7, 1966.
- Hider, R.C., Kong, X., 2010. Chemistry and biology of siderophores. *Nat Prod Rep* 27 (5), 637–657.
- Hiltner, L., 1904. About recent experiences and problems the field of soil bacteriology with special consideration of green manure and fallow. *Arbeiten der Deutschen Landwirtschaftlichen Gesellschaft* 98, 59–78.
- Hontzeasa, N., Zoidakisb, J., Glick, B.R., Abu-Omar, M.M., 2004. Expression and characterization of 1-aminocyclopropane-1-carboxylate deaminase from the rhizobacterium *Pseudomonas putida* UW4: a key enzyme in bacterial plant growth promotion. *Biochim Biophys Acta* 1703, 11–19.
- Inagaki, A.M., Guimarães, V.F., Lana, M.C., Jeferson Klein, J., Andréia Rodrigues da Costa, C.P., Rodrigues, L.F.O.S., et al., 2015. Maize initial growth with the inoculation of plant growth-promoting bacteria (PGPB) under different soil acidity levels. *Aust J Crop Sci* 9 (4), 271–280.
- Iqbal, A., Yabuta, Y., Takeda, T., Nakano, Y., Shigeoka, S., 2006. Hydroperoxide reduction by thioredoxin-specific glutathione peroxidase isoenzymes of *Arabidopsis thaliana*. *FEBS J* 273 (24), 5589–5597.
- Joe, M.M., Devara, S., Benson, A., Sa, T., 2016. Isolation of phosphate solubilizing endophytic bacteria from *Phyllanthus amarus* Schum & Thonn: evaluation of plant growth promotion and antioxidant activity under salt stress. *J Appl Res Med Aromat Plants* 3, 71–77.
- Karthikeyan, B., Joe, M.M., Islam, R., Sa, T., 2012. ACC deaminase containing diazotrophic endophytic bacteria ameliorate salt stress in *Catharanthus roseus* through reduced ethylene levels and induction of antioxidative defense systems. *Symbiosis* 56, 77–86.
- Kaushal, M., Wani, S.P., 2016. Plant-growth-promoting rhizobacteria: drought stress alleviators to ameliorate crop production in drylands. *Ann Microbiol* 66 (1), 35–42.
- Khan, A.L., Halo, B.A., Elyassi, A., Ali, S., Al-Hosni, K., Hussain, J., et al., 2016. Indole acetic acid and ACC deaminase from endophytic bacteria improves the growth of *Solanum lycopersicum*. *Electron J Biotechnol* 21, 58–64.
- Kloepper, J.W., Lifshitz, R., Zablotowicz, R.M., 1989. Free-living bacterial inocula for enhancing crop productivity. *Trends Biotechnol* 7, 39–43.
- Kloepper, J.W., Ryu, C.M., Zhang, S., 2004. Induced systemic resistance and promotion of plant growth by *Bacillus spp.* *Phytopathology* 94, 1259–1266.
- Kuan, K.B., Othman, R., Abdul Rahim, K., Shamsuddin, Z.H., 2016. Plant growth-promoting rhizobacteria inoculation to enhance vegetative growth, nitrogen fixation and nitrogen remobilisation of maize under greenhouse conditions. *PLoS One* 11 (3), e0152478.

- Kumar, A., Singh, R., Giri, D.D., Singh, P.K., Pandey, K.D., 2014. Effect of *Azotobacter chroococcum* CL13 inoculation on growth and curcumin content of turmeric (*Curcuma longa* L.). *Int J Curr Microbiol Appl Sci* 3 (9), 275–283.
- Kumar, A., Verma, H., Singh, V.K., Singh, P.P., Singh, S.K., Ansari, W.A., et al., 2017a. Role of *Pseudomonas* sp. in sustainable agriculture and disease management. In: Meena V., Mishra P., Bisht J., Pattanayak A. (Eds.), *Agriculturally Important Microbes for Sustainable Agriculture*. Springer, Singapore, pp. 195–215.
- Kumar, A., Vandana, R.S., Singh, M., Pandey, K.D., 2015c. Plant growth promoting rhizobacteria (PGPR). A promising approach for disease management. In: Singh, J.S., Singh, D.P. (Eds.), *Microbes and Environmental Management*. Studium Press, New Delhi, pp. 195–209.
- Kumar, A., Singh, R., Yadav, A., Giri, D.D., Singh, P.K., Pandey, K.D., 2016a. Isolation and characterization of bacterial endophytes of *Curcuma longa* L. *3 Biotech* 6, 60.
- Kumar, A., Singh, V., Singh, M., Singh, P.P., Singh, S.K., Singh, P.K., et al., 2016b. Isolation of plant growth promoting rhizobacteria and their impact on growth and curcumin content in *Curcuma longa* L. *Biocatal Agric Biotechnol* 8, 1–7.
- Kumar, A., Verma, H., Singh, V.K., Singh, P.P., Singh, S.K., Ansari, W.A., et al., 2017a. Role of *Pseudomonas* sp. in sustainable agriculture and disease management. In: Meena V., Mishra P., Bisht J., Pattanayak A. (Eds.), *Agriculturally Important Microbes for Sustainable Agriculture*. Springer, Singapore, pp. 195–215.
- Kumar, A., Singh, A.K., Kaushik, M.S., Mishra, S.K., Raj, P., Singh, P.K., et al., 2017b. Interaction of turmeric (*Curcuma longa* L.) with beneficial microbes: a review. *3 Biotech* 7 (6), 357.
- Kumar, P., Dubey, R.C., Maheshwari, D.K., 2012. *Bacillus* strains isolated from rhizosphere showed plant growth promoting and antagonistic activity against phytopathogens. *Microbiol Res* 167, 493–499.
- Kumar, V., Kumar, A., Pandey, K.D., Roy, B.K., 2015a. Isolation and characterization of bacterial endophytes from the roots of *Cassia tora* L. *Ann Microbiol* 65, 1391–1399.
- KVpczyńska, E., Zielińska, S., Kepczynski, J., 2003. Ethylene production by *Agrobacterium rhizogenes* strains in vitro and in vivo. *Plant Growth Regul* 39, 13–17.
- Lata, H., Li, X.C., Silva, B., Moraes, R.M., Halda-Alija, L., 2006. Identification of IAA-producing endophytic bacteria from micropropagated Echinacea plants using 16S rRNA sequencing. *Plant Cell Tissue Organ Cult* 85, 353–359.
- Liaqat, F., Eltem, R., 2016. Identification and characterization of endophytic bacteria isolated from in vitro cultures of peach and pear rootstocks. *3 Biotech* 6, 120.
- Lucy, M., Reed, E., Glick, B.R., 2004. Application of free living plant growth-promoting rhizobacteria. *Antonie Van Leeuwenhoek* 86, 1–25.
- Lutts, S., Kinet, J.M., Bouharmont, J., 1996. NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. *Ann Bot* 78, 389–398.
- Malusà, E., Pinzari, F., Canfora, L., 2016. Efficacy of biofertilizers: challenges to improve crop production. In: Singh D., Singh H., Prabha R. (Eds.), *Microbial Inoculants in Sustainable Agricultural Productivity*. Springer, India, pp. 17–40.
- Marques, A.P.G.C., Pires, C., Moreira, H., Rangel, A.O.S.S., Castro, P.M.L., 2010. Assessment of the plant growth promotion abilities of six bacterial isolates using *Zea mays* as indicator plant. *Soil Biol Biochem* 42, 1229–1235.
- Masood, A., Iqbal, N., Khan, N.A., 2012. Role of ethylene in alleviation of cadmium-induced photosynthetic capacity inhibition by sulphur in mustard. *Plant Cell Environ* 35, 524–533.
- Mazid, M., Khan, T.A., 2015. Future of bio-fertilizers in Indian agriculture: an overview. *Int J Agric Food Res* 3 (3), 10–23.

- Mazid, M., Khan, Z.H., Quddusi, S., Taqi, A.K., Firoz, M., 2011. Significance of sulphur nutrition against metal induced oxidative stress in plants. *J Stress Physiol Biochem* 7 (3), 65–84.
- McLaughlin, A., Mineau, P., 1995. The impact of agricultural practices on biodiversity. *Agric Ecosyst Environ* 55 (3), 201–212.
- McRose, D.L., Baars, O., Morel, F.M.M., Kraepiel, A.M.L., 2017. Siderophore production in *Azotobacter vinelandii* in response to Fe-, Mo- and V-limitation. *Environ Microbiol* 19 (9), 3595–3605.
- Miller, S.H., Browne, P., Prigent-Cambaret, C., Combes-Meynet, E., Morrissey, J.P., O’Gara, F., 2010. Biochemical and genomic comparison of inorganic phosphate solubilisation in *Pseudomonas* species. *Environ Microbiol Rep* 2, 403–411.
- Miranda, M.V.C., 2012. Effects of Microbial Inoculants on Biocontrol and Plant Growth Promotion. Ohio State University, Doctoral dissertation.
- Mittler, R., Vanderauwera, S., Gollery, M., Van Breusegem, F., 2004. Reactive oxygen gene network of plants. *Trends Plant Sci* 9 (10), 490–498.
- Montañez, A., Abreu, C., Gill, P.R., Hardarson, G., Siracdi, M., 2009. Biological nitrogen fixation in maize (*Zea mays* L.) by ¹⁵N isotope-dilution and identification of associated culturable diazotrophs. *Biol Fert Soils* 45, 253–263.
- Navarro-Noyaa, Y.E., Hernández-Mendozaa, E., Morales-Jiménez, J., Jan-Robleroa, J., Martínez-Romero, E., Hernández-Rodríguez, C., 2012. Isolation and characterization of nitrogen fixing heterotrophic bacteria from the rhizosphere of pioneer plants growing on mine tailings. *Appl Soil Ecol* 62, 52–60.
- Nazar, R., Khan, M.I.R., Iqbal, N., Masood, A., Khan, N.A., 2014. Involvement of ethylene in reversal of salt-inhibited photosynthesis by sulfur in mustard. *Physiol Plant* 152, 331–344.
- Okon, Y., Labandera-Gonzalez, R., 1994. Agronomic applications of *Azospirillum* inoculated roots. *Plant Soil* 90, 1591–1601.
- Oku, S., Komastu, A., Tajima, T., Nakashimada, Y., Kato, J., 2012. Identification of chemotaxis sensory proteins for aminoacids in *Pseudomonas fluorescens* Pf0-1 and their involvement in chemo taxis to tomato root exudates and root colonization. *Microbes Environ* 27 (4), 462–469.
- Olanrewaju, O.S., Glick, B.R., Babalola, O.O., 2017. Mechanisms of action of plant growth promoting bacteria. *World J Microbiol Biotechnol* 33 (11), 197.
- Orhan, F., 2016. Alleviation of salt stress by halotolerant and halophilic plant growth-promoting bacteria in wheat (*Triticum aestivum*). *Braz J Microbiol* 47, 621–627.
- Oteino, N., Lally, R.D., Kiwanuka, S., Lloyd, A., Ryan, D., Germaine, K.J., et al., 2015. Plant growth promotion induced by phosphate solubilizing endophytic *Pseudomonas* isolates. *Front Microbiol* 6, 745.
- Pandey, S.S., Singh, S., Babu, C.S.V., Shanker, K., Shrivastava, N.K., Kalra, A., 2016. Endophytes of opium poppy differentially modulate host plant productivity and genes for the biosynthetic pathway of benzylisoquinoline alkaloids. *Planta* 243, 1097–1114.
- Pierik, R., Tholen, D., Poorter, H., Visser, E.J., Voesenek, L.A.C.J., 2006. The Janus face of ethylene: growth inhibition and stimulation. *Trends Plant Sci* 11, 176–183.
- Pieterse, C.M., Zamioudis, C., Berendsen, R.L., Weller, D.M., Van Wees, S.C., Bakker, P.A., 2014. Induced systemic resistance by beneficial microbes. *Ann Rev Phytopathol* 52, 347–375.
- Pirlak, L., Kose, M., 2009. Effects of plant growth promoting rhizobacteria on yield and some fruit properties of strawberry. *J Plant Nutr* 32 (7), 1173–1184.
- Piromyong, P., Buranabanyat, B., Tantasawat, P., Tittabutr, P., Boonkerd, N., Teamroong, N., 2011. Effect of plant growth promoting rhizobacteria (PGPR) inoculation on microbial community structure in rhizosphere of forage corn cultivated in Thailand. *Eur J Soil Biol* 47, 44–54.

- Premachandra, D., Hudek, L., Brau, L., 2016. Bacterial modes of action for enhancing of plant growth. *J Biotechnol Biomater* 6, 236.
- Reetha, S., Bhuvaneswari, G., Thamizhiniyan, P., Mycin, T.R., 2014. Isolation of indole acetic acid (IAA) producing rhizobacteria of *Pseudomonas fluorescens* and *Bacillus subtilis* and enhance growth of onion (*Allium cepa* L). *Int J Curr Microbiol Appl Sci* 3 (2), 568–574.
- Reid, M.S., 1995. Ethylene in plant growth, development, and senescence. In: Davies, P.J. (Ed.), *Plant Hormones*. Springer, Dordrecht.
- Rijavec, T., Lapanje, A., 2016. Hydrogen cyanide in the rhizosphere: not suppressing plantpathogens, but rather regulating availability of phosphate. *Front Microbiol* 7, 1785.
- Sabat, S., Murthy, V.K., Shantha, S.L., Kushnoor, D., Agarwal, G., Thomas, J., et al., 2014. Comparative study of cytokinin production isolated from bacteria and shoot induction. *Indian J Biotechnol* 13 (4), 544–546.
- Saharan, B.S., Nehra, V., 2011. Plant growth promoting rhizobacteria: a critical review. *Life Sci Med Res* 21 (1), 30.
- Salomon, M.V., Bottini, R., de Souza Filho, G.A., Cohen, A.C., Moreno, D., Gil, M., et al., 2014. Bacteria isolated from roots and rhizosphere of *Vitis vinifera* retard water losses, induce abscisic acid accumulation and synthesis of defense-related terpenes in in vitro cultured grapevine. *Physiol Plant* 151 (4), 359–374.
- Sandhya, V., Shrivastava, M., Ali, S.Z., Prasad, V.S.K., 2017. Endophytes from maize with plant growth promotion and biocontrol activity under drought stress. *Russ Agric Sci* 43, 22–34.
- Sasirekha, B., Srividya, S., 2016. Siderophore production by *Pseudomonas aeruginosa* FP6, a biocontrol strain for *Rhizoctonia solani* and *Colletotrichum gloeosporioides* causing diseases in chilli. *Agric Nat Resour* 50, 250e256.
- Sev, T.M., Khai, A.A., Aung, A., Yu, S.S., 2016. Evaluation of endophytic bacteria from some rice varieties for plant growth promoting activities. *J Sci Innov Res* 5 (4), 144–148.
- Shahzad, R., Khan, A.L., Bilal, S., Waqas, M., Kang, S.M., Lee, I.J., 2017. Inoculation of abscisic acid-producing endophytic bacteria enhances salinity stress tolerance in *Oryza sativa*. *Environ Exp Bot* 136, 68–77.
- Singh, M., Kumar, A., Singh, R., Pandey, K.D., 2017c. Endophytic bacteria: a new source of bioactive compounds. *3 Biotech* 7 (5), 315.
- Singh, R., Pandey, D.K., Kumar, A., Singh, M., 2017a. PGPR isolates from the rhizosphere of vegetable crop *Momordica charantia*: characterization and application as bio-fertilizer. *Int J Curr Microbiol Appl Sci* 6 (3), 1789–1802.
- Singh, V.K., Singh, A.K., Kumar, A., 2017b. Disease management of tomato through PGPB: current trends and future perspective. *3 Biotech* 7 (4), 255.
- Smith, S.E., David, R., 2008. Introduction, In *Mycorrhizal Symbiosis*, third ed. Academic Press, London, 9780123705266.
- Sofo, A., Scopa, A., Nuzzaci, M., Vitti, A., 2015. Ascorbate peroxidase and catalase activities and their genetic regulation in plants subjected to drought and salinity stresses. *Int J Mol Sci* 16, 1356–13578.
- Souza, R.D., Ambrosini, A., Passaglia, L.M., 2015. Plant growth-promoting bacteria as inoculants in agricultural soils. *Genet Mol Biol* 38 (4), 401–419.
- Taghavi, S., Garafola, C., Monchy, S., Newman, L., Hoffman, A., Weyens, N., et al., 2009. Genome survey and characterization of endophytic bacteria exhibiting a beneficial effect on growth and development of poplar trees. *Appl Environ Microbiol* 75, 748–757.
- Thompson, J.E., Froese, C.D., Madey, E., Smith, M.D., Hong, Y.W., 1998. Lipid metabolism during plant senescence. *Prog Lipid Res* 37, 119–141.

- Timmusk, S., Behers, L., Muthoni, J., Muraya, A., Aronsson, A.C., 2017. Perspectives and challenges of microbial application for crop improvement. *Front Plant Sci* 8, 49.
- Tiwari, S., Lata, C., Chauhan, P.S., Nautiyal, C.S., 2016. *Pseudomonas putida* attunes morphophysiological, biochemical and molecular responses in *Cicer arietinum* L. during drought stress and recovery. *Plant Physiol Biochem* 99, 108e117.
- Tuomi, T., Rosenquist, H., 1995. Detection of abscisic, gibberellic and indole-3-acetic acid from plant and microbes. *Plant Physiol Biochem* 33, 725–734.
- Turan, M., Ataoglu, N. and Sezen, Y., 2004, October. Effects of phosphorus solubilizing bacteria (*Bacillus megaterium*) on yield and phosphorus contents of tomato plant (*Lycopersicon esculentum* L.) III. In: Proceedings of the National Fertilizer Congress. Farming—Industry—Environment, Tokat, Turkey (pp. 11–13).
- Vejan, P., Rosazlin, A., Tumirah, K., Salmah, I., Amru, N.B., 2016. Role of plant growth promoting rhizobacteria in agricultural sustainability—a review. *Molecules* 21, 573.
- Venkat, K.S., Soumya, M.S., Agarwal, H., Divya, G.D., 2017. Characterization and optimization of bacterium isolated from soil samples for the production of siderophores. *Resour Effic Technol* 3, 434–439.
- Verma, V.C., Singh, S.K., Prakash, S., 2011. Bio-control and plant growth promotion potential of siderophore producing endophytic Streptomyces from *Azadirachta indica*. *A Juss. J Basic Microbiol* 51, 550–556.
- Vessey, J.K., 2003. Plant growth promoting rhizobacteria as biofertilizers. *Plant Soil* 255, 571–586.
- Waadt, R., Hsu, P.K., Schroeder, J.I., 2015. Absciscic acid and other plant hormones: methods to visualize distribution and signaling. *Bioessays* 37 (12), 1338–1349.
- Wagner, E., Luche, S., Penna, L., Chevallet, M., Van Dorsselaer, A., Leize-Wagner, E., et al., 2002. A method for detection of overoxidation of cysteines: peroxiredoxins are oxidized in vivo at the active-site cysteine during oxidative stress. *Biochem J* 366 (3), 777–785.
- Wandersman, C., Delepelaire, P., 2004. Bacterial iron sources: from siderophores to hemophores. *Ann Rev Microbiol* 58, 611–647.
- Wang, D., Lv, S., Jiang, P., Li, Y., 2017. Roles, regulation, and agricultural application of plant phosphate transporters. *Front Plant Sci* 8, 817.
- Wang, X.M., Yang, B., Ren, C.G., Wang, H.W., Wang, J.Y., Dai, C.C., 2015. Involvement of abscisic acid and salicylic acid in signal cascade regulating bacteria. *Plant* 153, 30–42.
- Wani, S.H., Vinay, K., Varsha, S., Saroj, K.S., 2016. Phytohormones and their metabolic engineering for abiotic stress tolerance in crop plants. *Crop J* 4, 162–176.
- Willekens, H., Inzé, D., Van Montagu, M., Van Camp, W., 1995. Catalases in plants. *Mol Breed* 1 (3), 207–228.
- Yasmin, S., Zaka, A., Imran, A., Zahid, M.A., Yousaf, S., Rasul, G., et al., 2016. Plant growth promotion and suppression of bacterial leaf blight in rice by inoculated bacteria. *PLoS One* 11 (8), e0160688.
- Youssef, M.M.A., Eissa, M.F.M., 2014. Biofertilizers and their role in management of plant parasitic nematodes. A review. *E3J Biotechnol Pharm Res* 5 (1), 1–6.
- Yu, X., Ai, C., Xin, L., Zhou, G., 2011. The siderophore-producing bacterium, *Bacillus subtilis* CAS15, has a biocontrol effect on Fusarium wilt and promotes the growth of pepper. *Eur J Soil Biol* 47, 138e145.
- Zakry, F.A.A., Shamsuddin, Z.H., Rahim, K.A., Zakaria, Z.Z., Rahim, A.A., 2012. Inoculation of *Bacillus sphaericus* UPMB-10 to young oil palm and measurement of its uptake of fixed nitrogen using the ¹⁵N isotope dilution technique. *Microbe Environ* 27 (3), 257–262.
- Zandi, P., Basu, S.K., 2016. Role of Plant Growth-Promoting Rhizobacteria (PGPR) as bioFertilizers in stabilizing agricultural ecosystems. In: Nandwani, D. (Ed.), *Organic*

- Farming for Sustainable Agriculture. Sustainable Development and Biodiversity, Vol. 9. Springer, Cham.
- Zhou, C., Fi, L., Xie, Y., Zhu, L., Xiao, X., Ma, Z., et al., 2017. Involvement of abscisic acid in microbe-induced saline-alkaline resistance in plants. *Plant Signal Behav* 12 (10), 1143.

FURTHER READING

- Ndeddy Aka, R.J., Babalola, O.O., 2016. Effect of bacterial inoculation of strains of *Pseudomonas aeruginosa*, *Alcaligenes feacalis* and *Bacillus subtilis* on germination, growth and heavy metal (Cd, Cr, and Ni) uptake of *Brassica juncea*. *Int J Phytoremed* 18 (2), 200–209.
- Zeller, S.L., Brand, H., Schmid, B., 2007. Host-Plant Selectivity of Rhizobacteria in a Crop/Weed Model System. *Plos One* 2 (9), 846.

PGPR Bioelicitors: Induced Systemic Resistance (ISR) and Proteomic Perspective on Biocontrol

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4.1 INTRODUCTION

Physiological disorder in the plant leads to infectious diseases, have been considered as devastating menace to food security for the ever growing world population (Carvalho, 2006). Therefore, analysis of adequate amounts of balanced and secure foods that are produced in an environmentally feasible way is crucial (Carvalho, 2006). Indiscriminant application of pesticides is the baseline for intensive agriculture that resulted significant increases in crop yields for most field-grown fruit and vegetables crops as well. However, it is evident that many pesticides when used legitimately even after the instruction ignorance, may contaminate the food crops and soil ecosystems (E.E.A, 2005). Additionally, it is harmful to the beneficial soil microflora and creates residual problems. So, the biological control as alternatives can be very eco-friendly, efficacy used for the management of plant disease (Baysal et al., 2013).

Biological control is process in which pathogenic strain is maintained at low inoculum density either through one or more organisms' occurred naturally or by influencing the environment, host, or by introduction of one or more antagonists in mass (Saraf et al., 2014). In this regard, plant growth promoting rhizobacteria (PGPR) are beneficial groups that induce

plant defense mechanisms and provide more resistant to the host for further pathogen attack through highly diverse mechanisms. PGPR as biocontrol agents (BCAs) are more advantageous over regular chemical compounds as they are nonpathogenic, and naturally rhizosphere inhabitant, environmentally friendly, and directly promote plant growth. Moreover, their application is sustainable from an ecological perspective. Besides, PGPR possess diverse ranges of defense actions in plants that include antibiosis, production of siderophores, cell wall degrading enzymes (CWDEs), bio-surfactants and volatiles, induced systemic resistance (ISR), etc. (Fig. 4.1; Saraf et al., 2014).



4.2 PGPR AS BCAS AND THEIR MODE OF ACTIONS

For the fruitful biocontrol strategy, it is crucial to review the mode of action of the BCAs under different conditions.

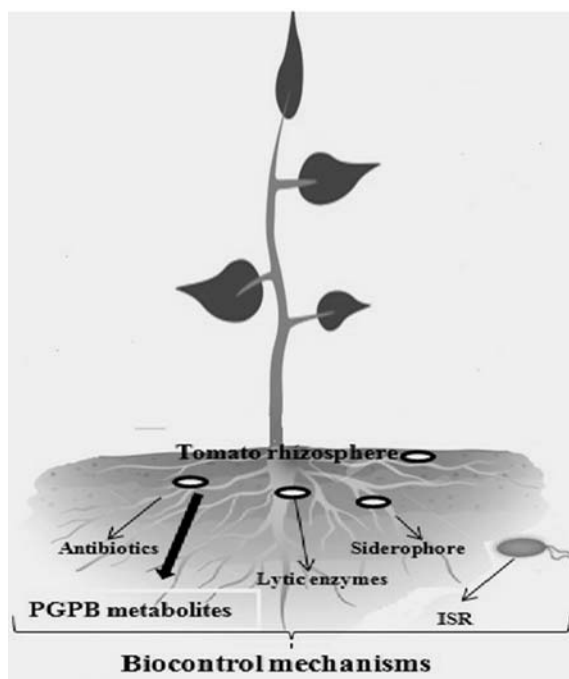


Figure 4.1 Biocontrol mechanisms of PGPR.

4.2.1 Antibiotics

Antibiosis is a widely recognized and effective biocontrol approach deployed by numerous PGPR for controlling the soil-borne infections in various crops (Handelman and Stabb, 1996). Different interactions shown between multiple groups of soil organisms are common, such as predation and competition for resources, antibiotics such as compounds (2,4-Diacetyl phloroglucinol, phenazine-1-carboxylic acid, phenazine-1-carboxamide, pyoluteorin, pyrrolnitrin, etc.,) are involved (Haas and Défago, 2005). It is evident that antibiotics produced by PGPR either lower down or check the soil-borne infections of wheat, rice, maize, chickpea, and barley (Raaijmakers et al., 2002). It was noticed that these antibiotics disrupt the pathogen membrane such as *Pythium* sp., thus, prevent the zoospores formation (de Souza et al., 2003). While, the phenazines not only impede the electron transport in target pathogen but also cause lipid damage and other macromolecules as well (Haas and Défago, 2005).

Pseudomonas genetic studies revealed that direct relationship disease suppression and antibiotic production (Vincent et al., 1991). Later studies indicated growing populations of *Pseudomonas* sp., [which produce the antibiotic 2,4-diacetylphloroglucinol (2,4- DAPG)], suppress the take-all disease (Raaijmakers and Weller 1998; de Souza et al., 2003). Phenazine-1-carboxylic acid (PCA) antibiotics produced by *P. fluorescens* and *P. aureofaciens* strains have also been found effective suppression of take-all in wheat seeds (Weller, 2007).

Apart from *Pseudomonas*, efficiency of *Bacillus* sp. as BCAs in various agricultural crops including the cereals has been extensively implemented in agricultural crops (Ashwini et al., 2014). *Bacillus* sp. offer several advantages over other BCAs as their endospore forming capacity can cope up osmotic conditions, extreme pH, and temperature. Earlier research reported the root surface colonizing activity of *Bacillus* sp. indirectly promote the plant growth and loss of fungal mycelia (Podile and Prakash 1996; Takayanagi et al., 1991). In context of biocontrol of *Bacillus* sp. Kim et al. (1997), isolated *Bacillus* sp. L324-92, and noted suppression action against take-all, root rot caused by *Rhizoctonia solani*, *Pythium irregulare*, and *P. ultimum*. Further studies revealed *Bacillus* sp., as a potent BCA in controlling root rot caused by fungal pathogens in field conditions (El-Meleigi et al., 2007). Similarly, application of *B. amyloliquifaciens* as seed treatment is known to suppress root rot caused by *Fusarium*

verticilloides in cereal crops such as maize and sorghum, respectively (Pereira et al., 2009; Idris et al., 2008). Besides, *Paenibacillus brasilensis* PB177, isolated from the maize rhizosphere in Brazil could be a potential BCA (von der Weid et al., 2005). Overall, reported research work demonstrated BCAs producing antibiotics have antimicrobial properties that have the potential to suppress the plant infecting fungal pathogens.

4.2.2 Siderophores

Siderophore compounds (molecular weight 400–1, 500 Da) perform antibiosis by providing iron to plants, thus making pathogens deprived of iron (Kloepper et al., 1980; O'sullivan and O'Gara, 1992; Maksimov et al., 2011). Deficiency in iron inhibits limit the growth of pathogens by blocking key processes such as, nucleic acid synthesis and sporulation (Mathiyazhagan et al., 2004).

Siderophores producing *Pseudomonas* has been the center of biological control since long time. Available literature revealed that fluorescent *Pseudomonas* sp., produce two major types of siderophores–pseudobactins (the fluorescent pigmented pyoverdins) (Lemanceau et al., 1993) and the pyochelins (nonfluorescent siderophore) (Leeman et al., 1996). These siderophores are widely known to promote plants growth and biocontrol of fungal diseases associated with several crops (Battu and Reddy, 2009). In this series, siderophores produced by *Pseudomonas* strain B324 in the suppression of *Pythium* root rot of wheat is important to discuss (Becker and Cook, 1988). Similarly, pyoverdine (another type of siderophore) produced by *Pseudomonas* were reported to protect against *Fusarium oxysporum* that cause wilt disease of potato (Schippers et al., 1987). In *Oryza sativa*, siderophore producing bacteria (*Bacillus*, *Kochuria*, and *Pseudomonas*) showed strong biological control against phytopathogens such as *F. oxysporum*, *Pyricularia oryzae*, and *Sclerotium* sp. (Chaiharin et al., 2009). Loper and Henkels (1999) observed less pyoverdins production and fungal pathogen suppression by mutants compared to the parental strains. Therefore, it is widely accepted that siderophore production is one of the important mechanisms of biological control.

4.2.3 Cell wall degrading enzymes

Production and secretion of CWDEs are the important aspect of BCAs to prevent the growth of soil-borne pathogens (Singh et al., 2017). CWDEs mode of action of is to disturb the structural integrity of target pathogens

cell wall (Budi et al., 2000). Plant pathogens, β -1,4-N-acetyl-glucoseamine, and chitin are the chief component of fungal pathogens cell wall. Therefore, available literature observed the role of several CWDEs such as, β -1,3-glucanase- and chitinase in most of BCAs. Such CWDEs generally involved lysis of cell wall and thus neutralize the inhibitory action of the pathogens (Goswami et al., 2016). In *S. marcescens*B2, the chitinolytic and antifungal activities were observed against the soil-borne pathogens *R. solani* and *F. oxysporum* (Someya et al., 2000). Extended work observed that the mycelia of the target fungal pathogens when co-inoculated with this strain witnessed various abnormalities such as partial swelling in the hyphae and at the tip, hyphal curling, or bursting of the hyphal tip. Another biocontrol process observed by BCAs deploying CWDEs against phytopathogenic infection includes control of *Sclerotium rolfsii* and *F. oxysporum* on beans (Felse and Panda, 2000). The β -1, 3-glucanase synthesized by strains of *Paenibacillus* and *Streptomyces* sp. lyse fungal cell walls of pathogenic *F. oxysporum*. In a similar manner, *Bacillus cepacia* synthesizes β -1,3-glucanase, which destroys the cell walls of the soilborne pathogens *R. solani*, *P. ultimum*, and *S. rolfsii* (Compant et al., 2005). Potential BCAs with chitinolytic activities include *B. licheniformis*, *B. cereus*, *B. circulans*, and *B. thuringiensis* (Sadfi et al., 2001). Among the gram-negative bacteria, *Serratia marcescens*, *Enterobacter agglomerans*, *Pseudomonas aeruginosa*, and *P. fluorescens* have been found to have chitinolytic activities (Nelson and Sorenson, 1999). Notorious phytopathogens such as, *Phytophthora capsici* and *Rhizoctonia solani* are also found inhibited by PGPR (Islam et al., 2016).

4.2.4 Volatile organic compounds

Volatile organic compounds (VOCs) are continuously synthesized and released into the atmosphere by anthropogenic and biogenic sources (originated from animals, microbes, and plants). VOCs originated from animals and plants are well studied compared to the microbial VOCs. Microbial VOCs are characterized as low molecular weight ($<300 \text{ g mol}^{-1}$) and with enough high vapor pressure under normal conditions that are produced through catabolic pathways, including glycolysis, proteolysis, and lipolysis (Schulz and Dickschat, 2007). Studies to date have acknowledged more than 200 variety of mVOCs released by various bacteria that include 75 fatty acid derivatives, 50 aromatic compounds, 74 nitrogen-containing compounds, 30 sulfur compounds, 96

terpenoids, and 18 compounds containing halogen, selenium, tellurium, or other metal (Schulz and Dickschat, 2007). In biocontrol, volatile of antagonistic microbes as a BCAs, receive more attentions because of eco-friendly, long-term security to crops against pathogens (Bhattacharyya and Jha, 2012). VOCs produced by BCAs have been reported for plant growth promotion, antimicrobial and nematocidal activity, and ISR in crops (Raza et al., 2013; Audrain et al., 2015). Species of *Bacillus* (Ryu et al., 2004a) and *Pseudomonas* (Han et al., 2006) have been well recognized for ISR through an ethylene pathway that protects tobacco (*Nicotiana spp.*), *A. thaliana* from *Pectobacterium carotovorum* and *Pseudomonas syringae* (Rudrappa et al., 2010). Similarly, Park et al. (2013) declared that exposed to C13 VOC emitted by *Paenibacillus polymyxa* E681 becomes resistant against *Pseudomonas syringae*.

4.2.5 Induced systemic resistance

Generally plants exhibit two types of resistance mechanisms depending upon the external stimuli- systemic acquired resistance (SAR) and ISR. SAR is executed upon the pathogen attack over plants, while ISR is switched on through colonization by PGPR (Van Loon et al., 1998; Kloepper and Beauchamp, 1992). SAR is executed through salicylic acid (SA) signal and any kind of obstacle in SA accumulation may impair SAR. On the other hand in ISR, PGPR colonization does not manifest any symptoms over host plant (Bakker et al., 2003). Molecular studies over *Arabidopsis* indicated that both SAR and ISR are connected through *NPR1* gene. Earlier literature suggested that in most of the cases, PGPR trigger ISR either by strengthening the structural integrity (physical and mechanical strength) of the cell wall or by altering the host physiological and biochemical reactions. This phenomenon leads to the production of defense chemicals signals such as chitinase, peroxidase, and proteinase inhibitors (Ramamoorthy et al., 2001; Nandakumar et al., 2001; Silva et al., 2004).

Published literature revealed diverse root-associated PGPR (*Pseudomonas* and *Bacillus sp.*) that excite the plant immune system primarily through Jasmonic acid-Ethylene (JA-ET) signaling path NRP-1 dependent manner (Pieterse et al., 2002; Van loon and Bakker, 2005) (Fig. 4.2). For instances, PGPR such as *Pseudomonas fluorescens* WCS417r have been shown to trigger ISR in several plant species and in *Arabidopsis* through JA/ET signaling pathways and in regulation with *NPR1* gene. JA

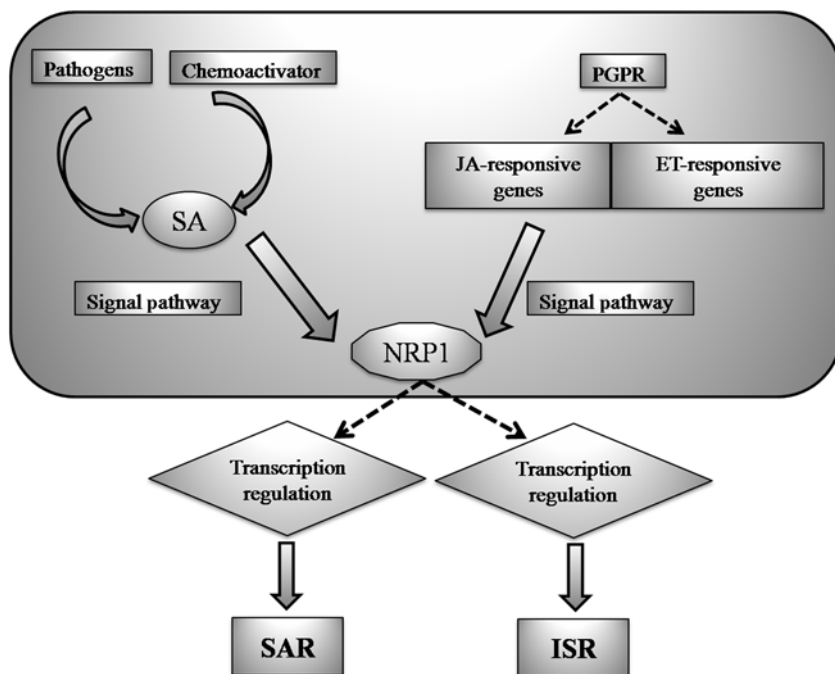


Figure 4.2 Signaling pathway of SAR and ISR.

signaling mutants such as, *jar1*, *jin1*, and *coi1* and diverse ET signaling mutants, including *etr1*, *ein2*, *ein3*, and *eir1*, were shown to be defective in *P. fluorescens* WCS417r–ISR. Such signaling pathway has also been reported in other PGPR strains, such as *Serratia marcescens* 90–166, *Pseudomonas protegens* CHA0, and *P. fluorescens* Q2–87, inducing ISR in *Arabidopsis* (Iavicoli et al., 2003; Ryu et al., 2004b). Though the expressions of various defense-related enzymes such as chitinases, b-1, 3-glucanase, peroxidase (PO), phenylalanine ammonia-lyase (PAL), and polyphenol oxidase (PPO), PGPR strains can also induce this type of systemic resistance in plants (Bharathi, 2004). In fact in some cases, PGPR mediated JA–ET dependent signaling overlaps the SA dependent pathways. Under short supply of iron, certain PGPR strains reported to produce SA as a siderophore (Meyer et al., 1992; Visca et al., 1993). Another study observed elicited resistance by *P. fluorescens* CHA0 in tobacco might be fully explained by the bacterial production of SA, indicating similarity of ISR signaling pathway with that of SAR signal transduction pathways.

ISR executed by PGPR has been widely noticed in array of plant species, (e.g., *Arabidopsis thaliana*, bean, carnation, cucumber, radish, tobacco,

and tomato) and significantly reduced the pathogenicity of series of plant pathogens, including fungi, bacteria, and viruses (Van Loon et al., 1998). Moreover, few compounds (elicitors) derived from bacteria have been reported to be the part of ISR (Van Loon et al., 1998; Bakker et al., 2003; Van loon and Bakker, 2005). ISR elicitors comprise cell wall components (lipopolysaccharides and flagella) as well as metabolites (siderophores and antibiotics such as 2,4-diacetylphloroglucinol) (Bakker et al., 2003; Van loon and Bakker, 2005; Iavicoli et al., 2003). In fact, mostly the ISR-accelerating PGPR acknowledged to date are gram-negative affiliated with *Pseudomonas*. However, *Bacillus* sp, such as *B. amyloliquefaciens*, *B. subtilis*, *B. pasteurii*, *B. cereus*, *B. pumilus*, *B. mycoides*, and *B. sphaericus* have gain momentum for ISR process against broad range of pathogens over the past decade. For instances, Huang et al. (2012) reported dimethyl disulfide (DMDS) produced by *B. cereus* strain, has ISR-eliciting activity against wide array of fungal pathogens. Recently, multiple elicitors have been detected in *B. amyloliquefaciens* SQR9 that act synergistically to induce ISR against different phytopathogens (*Pseudomonas syringae* Tomato DC3000 and *Botrytis cinerea*) through different signaling pathway genes (Wu et al., 2018). Recent progress in PGPR research reported that VOCs may play a key role in ISR. VOC (2,3-butanediol) synthesized by PGPR strains *B. subtilis* GB03 and *B. amyloliquefaciens* IN937a were considered to provoke plant defense responses (Ryu et al., 2004b). Additionally, the fengycins and surfactins derived from *B. subtilis* can also control plant diseases (Ongena et al., 2007).

Besides, endophytic bacterial groups are not lagging behind in ISR activity, compared to others. First indication of ISR activity was reported from *P. fluorescens* strain G8-4 (later on termed as 89B-61), that showed its negative response against cucumber anthracnose in cucumber. Another landmark and broad spectrum of ISR activity in cucumber was reported from *B. pumilus* INR7 that lower down the development of diseases such as angular leaf spot and cucurbit wilt. Besides, *B. pumilus* INR7 was also found resistant against pathogens such as cucumber mosaic virus (CMV), *Sclerotium rolfii*, *Ralstonia solanacearum*, *Colletotrichum gloeosporioides*, and *Rhizoctonia solani* (pepper and tomato), and the frequency of *Cronartium quercuum* f. sp. *fusiforme* causing Fusiform rust over loblolly pine (Wei et al., 1996; Enebak and Carey, 2000; Zehnder et al., 2001; Murphy et al., 2003). In *B. pumilus* SE34 ISR activity was amplified through structural barriers, production of toxic substances (e.g., phenolics and phytoalexins), accumulation of molecules (e.g., chitinase) and hydrolytic

enzymes (e.g., β -1,3-glucanases), which contribute in releasing oligosaccharides that results in stimulating other defense reactions. More or less the ISR control the pathogens over the outer region of the plant root cortex (Benhamou et al., 1996) by modifying the cell wall integrity (Benhamou et al., 1996, 1998). Such type of ISR was regulated by pathogenesis-related (PR) proteins, (chitinase and β -1,3-glucanase) and defense-related proteins, (peroxidase, PPO, phenylalanine ammonia-lyase) and phenolic compounds (Harish et al., 2009). Apart from fungal disease management, progressive research in endophytes highlighted ISR activity in plants to cope up viral pathogens. For instance, endophytes *B. subtilis* IN937b, *B. pumilus* SE34, and *B. amyloliquefaciens* IN937a were found to elicit ISR activity in tomato and protect against cucumber mosaic cucumovirus (CMV) (Zehnder et al., 2001). Similarly, reports of endophytes inducing ISR against banana bunchy top virus was also reported (Harish et al., 2009).



4.3 PROTEOMIC PERSPECTIVE ON BIOCONTROL

Research scenario on plant defense mechanisms including BCAs are changing with the advent of cutting edge molecular techniques such as, genomics and proteomics. Proteomics is comparatively recent technique, coined in 1994 and can be defined as the “the systematic analysis of the set of proteins in a specific cell, tissue or sub-cellular compartment.” It generates insight of particular process in instantly as well the external or internal factors governing the cellular process. Basically, proteomics deals with conventional techniques of two-dimensional gel electrophoresis (2-DGE) where the entire cellular proteins in the first phase are separated by isoelectric focusing (IEF). Furthermore, the proteins in the second phase are resolved on the basis of molecular charges under the influence of sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) (Westermeier and Naven, 2002; Simpson, 2003, 2004). Following steps are subjected for identification of separated proteins by comigration with known proteins through immunoblotting, N-terminal sequencing, peptide mass determination by mass spectroscopy (MS), peptide sequencing by tandem MS, and correlating the mass and sequence data with information in protein, genome, and expressed sequence tag (EST) databases (Graves and Haystead, 2002; Patton, 2002). Recently,

proteomics has been revolutionized by the protein microarray and the application of bioinformatics helps in resolving the myth of protein-protein interactions under specific conditions. However, progress has been found in replacing microarray technique with protein capture methods including the yeast two-hybrid (Y2H) systems or the isolation of proteins/protein complexes by affinity chromatography and other separation techniques. Y2H is an impressive technique of identifying protein-protein interactions (PPIs). The usefulness of this test is the revival of downstream reporter gene(s) by the fixing of a transcription factor onto an upstream activating sequence (UAS). Prerequisite condition for two-hybrid screening, the transcription factor is split into two separate fragments, called the binding domain (BD) and activating domain (AD). The BD is the domain responsible for binding to the UAS and the AD is the domain responsible for activation of transcription (Causier and Davies, 2002).

In biocontrol, the proteomic technique is more relevant compared to parallel techniques like genomics, to unravel set of expressed proteins that causes physiological, structural, and biochemical alterations during pre- or-post infection stages arise due to the host-pathogen interactions. Background studies revealed various types of proteins, metabolites, and toxins encoded by pathogens genes that are enough to establish disease in host plant which have been termed as pathogenicity and/or virulence factors. Compared to the conventional molecular studies, advanced proteomics and the bioinformatics approaches have contributed enough during the last decades to unravel the functions of virulent or pathogenesis factors of the phytopathogenic fungi (Acero et al., 2011).

However, information via proteomics for inducing plant protection using PGPR is limited, and to date few workers have identified and characterized novel or key proteins involved in plant defense mechanisms against pathogen infection (Table 4.1). Application of proteomics in PGPR can be beneficial in unraveling novel determinants that could define new biocontrol formulations with enhanced biocontrol potential.



4.4 CONCLUSION AND FUTURE PERSPECTIVE

In present scenario, PGPR research has been an encouraging initiative for sustainable agriculture as their multiple roles in favor of plant

Table 4.1 Glimpses of proteomics approaches used in PGPR for biocontrol activity

PGPR strains	Pathogen/insect pest	Proteomic approach	References
<i>Paenibacillus polymyxa</i> SQR-21; China	<i>Fusarium</i> sp.	Protein extraction and digestion; LC–MS	E et al. (2017)
<i>Paenibacillus polymyxa</i> NSY50	<i>Fusarium oxysporum</i>	TEM; 2D- MALDI-TOF/TOF analysis	Du et al. (2016)
<i>Bacillus</i> EU07, QST713 and FZB24	<i>Fusarium oxysporum</i>	2D- MALDI-TOF/TOF analysis; LC–MS	Baysal et al. (2013)
<i>Pseudomonas chlororaphis</i> O6; Korea	<i>Erwinia carotovora</i>	Protein Extraction; 2D	Kim et al. (2014)
<i>Paenibacillus polymyxa</i> E681; Korea	<i>Botrytis cinerea</i>	Protein extraction; 2D- MALDI-TOF/TOF analysis	Kwon et al. (2016)
<i>Pseudomonas fluorescens</i> WR-1; China	<i>Ralstonia solanacearum</i>	Protein extraction; 2D-MALDI	Raza et al. (2017)
<i>Bacillus amyloliquefaciens</i> NC6 I; China	<i>Botrytis cinerea</i> and Tobacco mosaic virus	Protein extraction; SDS-PAGE; MALDI	Wang et al. (2016)
<i>Bacillus subtilis</i> GB03; Korea	<i>Erwinia carotovora</i>	Protein extraction; 2D- MALDI-TOF/ TOF MS	Kwon et al. (2010)

productivity has been immensely tapped. As BCAs, PGPR has been widely accepted and gained tremendous momentum in safely controlling the plant diseases without disturbing the ecosystem. The beauty of this group is that they act as “stress reliever” under abiotic and biotic stress conditions. However, the success of biocontrol approach depends on certain factors, such as environmental conditions, inoculums concentration/ density and fidelity of host resistance, etc. Therefore, further research requires the application of mathematical modeling to measure the effects of abiotic and biotic environmental drivers on demography and population dynamics of real biological control systems in the field.

Additionally, futures work must be to exploitation of demanding technology such as, nanotechnology in biocontrol of plant diseases. *Pseudomonas* and *Bacillus* like PGPR are well known for producing

antimicrobial compounds. Application of nanotechnology may significantly reduce the pathogens growth, thus acting as potential nanoBCAs. Moreover, encapsulation of ant-toxins can be helpful in controlling pathogens.

Furthermore, biocontrol efficacy can be upgraded through transgenic technology in order to over express one or multiple antiphytopathogenic traits within one PGPR and can act collectively. Besides, in-depth studies of the tissue—specific microbiome is required to investigate the tripartite relation between the pathogen-host and PGPR. Besides, rhizospheric activity affecting factors (effect of cultivar, environmental conditions) are the major pinpoints of future research theme. An attempt to overcome problems of varying efficacy may be attained by strain mixing, improved inoculation techniques or gene transfer of active genetic source of antagonists to the host plant. The soil microbes are active elements for soil development and the basis of sustainable agriculture. Certainly, ISR mechanism of biocontrol in plants could revolutionize agriculture sector, nevertheless basic research on PGPR application and uses of modern tool and techniques to support plants from laboratory to field has been lacking till date.

Remarkable progress has been done in deployment of bioelicitors for biocontrol process, however, the scope of the VOC-derived technologies need to be disseminate in near future. Apart from, most of the volatiles showed their biocontrol efficiency in laboratory conditions. Thus, future perspectives of VOCs need open field trials study.

In addition, proteomic and genomic studies of BCAs will provide fundamental insights into the microbial ecology of the phytosphere (the environment immediately surrounding and including the plant), which encompasses the primary loci of biocontrol. Background decades highlighted the proteomic is limited to study the various biocontrol aspects of *Trichoderma* (cellular proteins, the inducement of secreted proteins in phytopathogenic fungal cell walls, mitochondria proteins, and three-way interactions among *Trichoderma atroviride*, plant, and fungal pathogens). Therefore, future research demands proteomic analysis of nonpathogenic rhizosphere organisms to define tripartite role of PGPR–host root cell modifications–pathogens in biocontrol activity.

REFERENCES

- Acero, F.J.F., Carbú, M., El-Akhal, M.R., Garrido, C., González-Rodríguez, V.E., Cantoral, J.M., 2011. Development of proteomics-based fungicides: New strategies for environmentally friendly control of fungal plant diseases. *Int. J. Mol. Sci.* 12, 795–816.
- Ashwini, N., Srividya, S., 2014. Potentiality of *Bacillus subtilis* as biocontrol agent for management of anthracnose disease of chilli caused by *Colletotrichum gloeosporioides* OGC1. *3 Biotech* 4, 127–136.
- Audrain, B., Frag, M.A., Ryu, C.M., Ghigo, J.M., 2015. Role of bacterial volatile compounds in bacterial biology. *FEMS Microbiol Rev* 39(2), 222–233.
- Bakker, P.A.H.M., Ran, L.X., Pieterse, C.M.J., Van Loon, L.C., 2003. Understanding the involvement of rhizobacteria-mediated induction of systemic resistance in biocontrol of plant diseases. *Can J Plant Pathol* 25 (1), 5–9.
- Battu, P.R., Reddy, M.S., 2009. Siderophore-mediated antibiosis of rhizobacterial fluorescent pseudomonads against rice fungal pathogens. *Int J Pharm Tech Res* 1 (2), 227–229.
- Baysal, Ö., Lai, D., Xu, H.H., Siragusa, M., Çalışkan, M., Carimi, F., et al., 2013. A proteomic approach provides new insights into the control of soil-borne plant pathogens by *Bacillus* species. *PloS One* 8 (1), e53182.
- Becker, J.O., Cook, R.J., 1988. Role of siderophore in suppression of *Pythium* species and production of increased growth response of wheat by fluorescent *Pseudomonas*. *Phytopathology* 78, 778–782.
- Benhamou, N., Kloepper, J.W., Quadt-Hallman, A., Tuzun, S., 1996. Induction of defense-related ultrastructural modifications in pea root tissues inoculated with endophytic bacteria. *Plant Physiol* 112(3), 919–929.
- Benhamou, N., Kloepper, J.W., Tuzun, S., 1998. Induction of resistance against *Fusarium* wilt of tomato by combination of chitosan with an endophytic bacterial strain: ultrastructure and cytochemistry of the host response. *Planta* 204 (2), 153–168.
- Bharathi, S., 2004. Development of botanical formulations for the management of major fungal diseases of tomato and onion. Tamil Nadu Agricultural University, Coimbatore, India, p. 152, PhD Thesis.
- Bhattacharyya, P.N., Jha, D.K., 2012. Plant growth-promoting rhizobacteria (PGPR): Emergence in agriculture. *World J. Microbiol. Biotechnol.* 28, 1327–1350.
- Budi, S.W., van Tuinen, D., Arnould, C., Dumas-Gaudot, E., Gianinazzi-Pearson, V., Gianinazzi, S., 2000. Hydrolytic enzyme activity of *Paenibacillus* sp. strain B2 and effects of the antagonistic bacterium on cell integrity of two soil borne pathogenic bacteria. *Appl Soil Ecol* 15, 191–199.
- Carvalho, F.P., 2006. Agriculture, pesticides, food security and food safety. *Environ Sci Policy* 9 (7), 685–692.
- Causier, B., Davies, B., 2002. Analysing protein-protein interactions with the yeast two-hybrid system. *Plant Mol Biol* 50 (6), 855–870.
- Chaiharn, M., Chunhaleuchanon, S., Lumyong, S., 2009. Screening siderophore producing bacteria as potential biological control agent for fungal rice pathogens in Thailand. *World J Microbiol Biotechnol* 25(11), 1919–1928.
- Compant, S., Duffy, B., Nowak, J., Clément, C., Barka, E.A., 2005. Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. *Appl Environ Microbiol* 71 (9), 4951–4959.
- de Souza, J.T., Weller, D.M., Raaijmakers, J.M., 2003. Frequency, diversity and activity of 2, 4-diacetylphloroglucinol producing fluorescent *Pseudomonas* spp. in Dutch take-all decline soils. *Phytopathology* 93(1), 54–63.
- Du, N., Shi, L., Yuan, Y., Li, B., Shu, S., Sun, J., et al., 2016. Proteomic analysis reveals the positive roles of the plant-growth-promoting rhizobacterium NSY50 in the

- response of cucumber roots to *Fusarium oxysporum* f. sp. *cucumerinum* inoculation. *Front Plant Sci* 7, 1859.
- E.E.A., 2005. Environment and Health, European Environment Agency, EEA Report No.10.
- El-Meleigi, M.A., Hassen, Z.M., Ibrahim, G.H., 2007. Biological control of common root rot of spring wheat by coating seeds with *Bacillus* or *Trichoderma* spp. *JKUA, Meteorol Environ Arid Agric Sci* 18, 3–12.
- Enebak, S.A., Carey, W.A., 2000. Evidence for induced systemic protection to fusiform rust in loblolly pine by plant growth-promoting rhizobacteria. *Plant Dis* 84 (3), 306–308.
- Felse, P.A., Panda, T., 2000. Production of microbial chitinases—a revisit. *Bioprocess Biosyst Eng* 23 (2), 127–134.
- Goswami, D., Thakkar, J.N., Dhandhukia, P.C., 2016. Portraying mechanics of plant growth promoting rhizobacteria (PGPR): a review. *Cogent Food Agric* 2(1), 1127500.
- Graves, P.R., Haystead, T.A., 2002. Molecular biologist's guide to proteomics. *Microbiol Mol Biol Rev* 66 (1), 39–63.
- Haas, D., Défago, G., 2005. Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nat Rev Microbiol* 3 (4), 307–319.
- Han, S.H., Lee, S.J., Moon, J.H., Park, K.H., Yang, K.Y., Cho, B.H., et al., 2006. GacS-dependent production of 2R, 3R-butanediol by *Pseudomonas chlororaphis* O6 is a major determinant for eliciting systemic resistance against *Erwinia carotovora* but not against *Pseudomonas syringae* pv. *tabaci* in tobacco. *Mol PlantMicrobe Interact* 19 (8), 924–930.
- Handelman, J., Stabb, E.V., 1996. Biocontrol of soilborne plant pathogens. *Plant Cell* 8 (10), 1855.
- Harish, S., Kavino, M., Kumar, N., Balasubramanian, P., Samiyappan, R., 2009. Induction of defense-related proteins by mixtures of plant growth promoting endophytic bacteria against banana Bunchy top virus. *Biol Control* 5, 16–25.
- Huang, C.J., Tsay, J.F., Chang, S.Y., Yang, H.P., Wu, W.S., Chen, C.Y., 2012. Dimethyl disulfide is an induced systemic resistance elicitor produced by *Bacillus cereus* C1L. *Pest Manag Sci* 68 (9), 1306–1310.
- Iavicoli, A., Boutet, E., Buchala, A., Métraux, J.P., 2003. Induced systemic resistance in *Arabidopsis thaliana* in response to root inoculation with *Pseudomonas fluorescens* CHA0. *Mol PlantMicrobe Interact*, 16(10), 851–858.
- Idris, H.A., Labuschagne, N., Korsten, L., 2008. Suppression of *Pythium ultimum* root rot of sorghum by rhizobacterial isolates from Ethiopia and South Africa. *Biol Control* 45 (1), 72–84.
- Islam, S., Akanda, A.M., Prova, A., Islam, Md. T., Hossain, Md. M., 2016. Isolation and identification of plant growth promoting rhizobacteria from cucumber rhizosphere and their effect on plant growth promotion and disease suppression. *Front Microbiol* 6 (1360), 1–12.
- Kim, C.H., Kim, Y.H., Anderson, A.H., Kim, A.C., 2014. Proteomic analysis of a global regulator GacS sensor kinase in the rhizobacterium. *Pseudomonas Chlororaphis* O6. *Plant Pathol J* 30 (2), 220–227.
- Kim, D.S., Cook, R.J., Weller, D.M., 1997. *Bacillus* sp. L324-92 for biological control of three root diseases of wheat grown reduced tillage. *Phytopathology* 87(5), 551–558.
- Kloepper, J.W., Beauchamp, C.J., 1992. A review of issues related to measuring colonization of plant roots by bacteria. *Can J Microbiol* 38 (12), 1219–1232.
- Kloepper, J.W., Leong, J., Teintze, M., Scroth, M.N., 1980. Enhancing plant growth by siderophores produced by plant growth promoting rhizobacteria. *Nature* 286, 885–886.

- Kwon, Y.S., Ryu, C.M., Lee, S., Park, H.B., Han, K.S., Lee, J.H., et al., 2010. Proteome analysis of *Arabidopsis* seedlings exposed to bacterial volatiles. *Planta* 232(6), 1355–1370.
- Kwon, Y.S., Lee, D.Y., Rakwal, R., Baek, S.B., Lee, J.H., Kwak, Y.S., et al., 2016. Proteomic analyses of the interaction between the plant-growth promoting rhizobacterium *Paenibacillus polymyxa* E681 and *Arabidopsis thaliana*. *Proteomics* 16(1), 122–135.
- Leeman, M., den Ouden, F.M., van Pelt, J.A., Dirkx, F.P.M., Steiji, H., Bakker, P.A.H.M., Schippers, B., 1996. Iron availability affects induction of systemic resistance to *Fusarium* wilt of radish by *Pseudomonas fluorescens*. *Phytopathology* 86, 149–155.
- Lemanceau, P., Bakker, P.A.H.M., De Kogel, W.J., Alabouvette, C., Schippers, B., 1993. Antagonistic effect of non pathogenic *Fusarium oxysporum* Fo47 and pseudobactin 358 upon pathogenic *Fusarium oxysporum* f. sp. *Dianthi*. *Appl Environ Microbiol* 59(1), 74–82.
- Loper, J.E., Henkels, M.D., 1999. Utilization of heterologous siderophores enhances level of iron available to *Pseudomonas putida* in the rhizosphere. *Appl Environ Microbiol* 65 (12), 5357–5363.
- Maksimov, I.V., Abizgil'Dina, R.R., Pusenkova, L.I., 2011. Plant growth promoting rhizobacteria as alternative to chemical crop protectors from pathogens. *Appl Biochem Microbiol* 47 (4), 333–345.
- Mathiyazhagan, S., Kavitha, K., Nakkeeran, S., Chandrasekar, G., Manian, K., Renukadevi, P., et al., 2004. PGPR mediated management of stem blight of *Phyllanthus amarus* (Schum and Thonn) caused by *Corynespora cassicola* (Berk and Curt) Wei. *Arch Phytopathol Plant Protect* 37 (3), 183–199.
- Meyer, J.M., Azelvandre, P., Georges, C., 1992. Iron metabolism in *Pseudomonas*: salicylic acid, a siderophore of *Pseudomonas fluorescens* CHA0. *Biofactors* 4(1), 23–27.
- Murphy, J.F., Reddy, M.S., Ryu, C.M., Kloepper, J.W., Li, R., 2003. Rhizobacteria-mediated growth promotion of tomato leads to protection against Cucumber mosaic virus. *Phytopathology* 93 (10), 1301–1307.
- Nandakumar, R., Babu, S., Viswanathan, R., Raguchander, T., Samiyappan, R., 2001. Induction of systemic resistance in rice against sheath blight disease by *Pseudomonas fluorescens*. *Soil Biol Biochem* 33 (4), 603–612.
- Nelson, M.N., Sorenson, J., 1999. Chitinolytic activity of *Pseudomonas fluorescens* isolates from barley and sugar beet rhizosphere. *FEMS Microbiol. Ecol.* 30, 217–227.
- Ongena, M., Jourdan, E., Adam, A., Paquot, M., Brans, A., Joris, B., et al., 2007. Surfactin and fengycin lipopeptides of *Bacillus subtilis* as elicitors of induced systemic resistance in plants. *Environ Microbiol* 9 (4), 1084–1090.
- O'sullivan, D.J., O'Gara, F., 1992. Traits of fluorescent *Pseudomonas* spp. involved in suppression of plant root pathogens. *Microbiol Rev* 56 (4), 662–676.
- Park, H.B., Lee, B., Kloepper, J.W., Ryu, C.M., 2013. One shot-two pathogens blocked: Exposure of *Arabidopsis* to hexadecane, a long chain volatile organic compound, confers induced resistance against both *Pectobacterium carotovorum* and *Pseudomonas syringae*. *Plant Signal Behav* 8 (7), e24619.
- Patton, W.F., 2002. Detection technologies in proteome analysis. *J Chromatogr B Analyt Technol Biomed Life Sci* 771 (1), 3–31.
- Pereira, P., Nesci, A., Etcheverry, M.G., 2009. Efficacy of bacterial seed treatments for the control of *Fusarium verticillioides* in maize. *Bio Control* 54 (1), 103–111.
- Pieterse, C.M.J., Van Wees, S.C.M., Ton, J., Van Pelt, J.A., Van Loon, L.C., 2002. Signalling in rhizobacteria-induced systemic resistance in *Arabidopsis thaliana*. *Plant Biol.* 4, 535–544.
- Podile, A.R., Prakash, A.P., 1996. Lysis and biological control of *Aspergillus niger* by *Bacillus subtilis* AF 1. *Can J Microbiol* 42 (6), 533–538.

- Raaijmakers, J.M., Weller, D.M., 1998. Natural plant protection by 2, 4-diacetylphloroglucinol-producing *Pseudomonas* spp. in take-all decline soils. *Mol Plant Microbe Interact* 11 (2), 144–152.
- Raaijmakers, J.M., Vlam, M., de Souza, J.T., 2002. Antibiotic production by bacterial biocontrol agent. *Anton van Leeuwenhoek* 81(1–4), 537–547.
- Ramamoorthy, V., Viswanathan, R., Raguchander, T., Prakasam, V., Samiyappan, R., 2001. Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pests and diseases. *Crop Protect* 20 (1), 1–11.
- Raza, W., Faheem, M., Yousaf, S., Rajer, F.U., Yamin, M., 2013. Volatile and nonvolatile antifungal compounds produced by *Trichoderma harzianum* SQR-T037 suppressed the growth of *Fusarium oxysporum* f. sp. Niveum. *Sci Lett* 1, 21–24.
- Raza, W., Ling, N., Liu, D., Wei, Z., Hwang, Q., Shen, Q., 2017. Volatile organic compounds produced by *Pseudomonas fluorescens* WR-1 restrict the growth and virulence traits of *Ralstonia solanacearum*. *Microbiol Res* 192, 103–113.
- Rudrappa, T., Biedrzycki, M.L., Kunjeti, S.G., Donofrio, N.M., Czymbek, K.J., Paul, W.P., et al., 2010. The rhizobacterial elicitor acetoin induces systemic resistance in *Arabidopsis thaliana*. *Commun Integr Biol* 3 (2), 130–138.
- Ryu, C.M., Farag, M.A., Hu, C.H., Reddy, M.S., Kloepper, J.W., Paré, P.W., 2004a. Bacterial volatiles induce systemic resistance in *Arabidopsis*. *Plant Physiol* 134 (3), 1017–1026.
- Ryu, C.M., Murphy, J.F., Mysore, K.S., Kloepper, J.W., 2004b. Plant growth-promoting rhizobacteria systemically protect *Arabidopsis thaliana* against Cucumber mosaic virus by a salicylic acid and NPR1-independent and jasmonic acid-dependent signaling pathway. *Plant J*, 39(3), 381–392.
- Sadfi, N., Cherif, M., Fliss, I., Boudabbous, A., Antoun, H., 2001. Evaluation of bacterial isolates from salty soils and *Bacillus thuringiensis* strains for the biocontrol of *Fusarium* dry rot of potato tubers. *J Plant Pathol* 83, 101–117.
- Saraf, M., Pandya, M., Thakkar, A., 2014. Role of allelochemicals in plant growth promoting rhizobacteria for biocontrol of phytopathogens. *Microbiol Res* 169(1), 18–29.
- Schippers, B., Bakker, A.W., Bakker, P.A.H., 1987. Interactions of deleterious and beneficial rhizosphere microorganisms and the effect of cropping practices. *Ann Rev Phytopathol* 25, 339–358.
- Schulz, S., Dickschat, J.S., 2007. Bacterial volatiles: the smell of small organisms. *Nat Prod Rep* 24 (4), 814–842.
- Silva, H.S.A., de Silva, R.R., Macagnan, D., de Almeda Halfeld-Viera, B., Pereira, M.C.B., Mounteer, A., 2004. Rhizobacterial induction of systemic resistance in tomato plants: non-specific protection and increase in enzyme activities. *Biol. Control* 29, 288–295.
- Simpson, R.J., 2003. *Proteins and Proteomics: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, New York, p. 926.
- Simpson, R.J., 2004. *Purifying Proteins for Proteomics: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, New York, p. 801.
- Singh, V.K., Singh, A.K., Kumar, A., 2017. Disease management of tomato through PGPR: current trends and future perspective. *3 Biotech* 7, 255–264.
- Someya, N., Kataoka, N., Komagata, T., Hirayae, K., Hibi, T., Akutsu, K., 2000. Biological control of cyclamen soilborne diseases by *Serratia marcescens* strain B2. *Plant Dis* 84 (3), 334–340.
- Takayanagi, T., Ajisaka, K., Takiguchi, Y., Shimahara, K., 1991. Isolation and characterization of thermostable chitinases from *Bacillus licheniformis* X-7u. *Biochim Biophys Acta* 1078 (3), 404–410.

- Van Loon, L.C., Bakker, P.A.H.M., 2005. Induced systemic resistance as a mechanism of disease suppression by rhizobacteria. PGPR: Biocontrol and Biofertilization. Springer, Netherlands, pp. 39–66.
- Van Loon, L.C., Bakker, P.A.H.M., Pieterse, C.M.J., 1998. Systemic resistance induced by rhizosphere bacteria. *Ann Rev Phytopathol* 36 (1), 453–483.
- Vincent, M.N., Harrison, L.A., Brackin, J.M., Kovacevich, P.A., Mukerji, P., Weller, D. M., et al., 1991. Genetic analysis of the antifungal activity of a soilborne *Pseudomonas aureofaciens* strain. *Appl Environ Microbiol* 57 (10), 2928–2934.
- Visca, P., Ciervo, A., Sanfilippo, V., Orsi, N., 1993. Iron-regulated salicylate synthesis by *Pseudomonas spp.* *J Gen Microbiol* 139, 1995–2001.
- von der Weid, I., Artursson, V., Seldin, L., Jansson, J.K., 2005. Antifungal and root surface colonization properties of GFP-tagged *Paenibacillus brasilensis* PB177. *World J Microbiol Biotechnol* 21 (8), 1591–1597.
- Wang, N., Liu, M., Guo, L., Yang, X., Qiu, D., 2016. A novel protein elicitor (peba1) from *Bacillus amyloliquefaciens* NC6 induces systemic resistance in tobacco. *Int J Biol Sci* 12 (6), 757–767.
- Wei, G., Kloepper, J.W., Tuzan, S., 1996. Induced systemic resistance to cucumber diseases and increased plant growth by plant growth-promoting rhizobacteria under field conditions. *Phytopathology* 86, 221–224.
- Weller, D.M., 2007. *Pseudomonas* biocontrol agents of soilborne pathogens: looking back over 30 years. *Phytopathology* 97 (2), 250–256.
- Westermeier, R., Naven, T., 2002. Proteomics in Practice: A Laboratory Manual of Proteome Analysis. Wiley-vch, Weinheim, pp. 11–98.
- Wu, G., Liu, Y., Xu, Y., Zhang, G., Shen, Q., Zhang, R., 2018. Exploring elicitors of the beneficial rhizobacterium *Bacillus amyloliquefaciens* SQR9 to induce plant systemic resistance and their interactions with plant signaling pathways. *Mol PlantMicrobe Interact* 31, 560–567. Available from: <https://doi.org/10.1094/MPMI-11-17-0273-R>.
- Zehnder, G.W., Murphy, J.F., Sikora, E.J., Kloepper, J.W., 2001. Application of rhizobacteria for induced resistance. *Europ J Plant Pathol* 107 (1), 39–50.

FURTHER READING

- Ahn, I.P., Park, K., Kim, C.H., 2002. Rhizobacteria-induced resistance perturbs viral disease progress and triggers defense-related gene expression. *Mol Cells* 13, 302–308.
- Bacon, C.W., Hinton, D.M., 2002. Endophytic and biological control potential of *Bacillus mojavensis* and related species. *Biolog Cont* 23, 274–284.
- Bacon, C.W., Hinton, D.M., Porter, J.K., Glenn, A.E., Kuldau, G.A., 2004. Fusaric acid, a *Fusarium verticillioides* metabolite, antagonistic to the endophytic biocontrol bacterium *Bacillus mojavensis*. *Can J Bot* 82, 878–885.
- Baker, K.F., Cook, J.R., 1974. Biological Control of Plant Pathogens. WH Freeman and Company, San Francisco.
- Bandow, J.E., Brötz, H., Leichert, L.I.O., Labischinski, H., Hecker, M., 2003. Proteomic approach to understanding antibiotic action. *Antimicrob Agents Chemother* 47 (3), 948–955.
- Bent, A.F., Yu, I.C., 1999. Applications of molecular biology to plant disease and insect resistance. *Advan Agron* 66, 251–298.
- Bland, J.M., 1996. The first synthesis of a member of the iturin family, the antifungal cyclic lipopeptide, iturin-A2. *J Org Chem* 61, 5663–5664.
- Bouizgarne, B., El-Maarouf-Bouteau, H., Madiona, K., Biligui, B., Monestiez, M., Pennarun, A.M., et al., 2006. A putative role for fusaric acid in biocontrol of the parasitic angiosperm *Orobancha ramosa*. *Mol Plant Microbe Interact* 19 (5), 550–556.

- Chen, C., Bauske, E.M., Musson, G., Rodríguez-Kábana, R., Kloepper, J.W., 1995. Biological control of *Fusarium* wilt of cotton by use of endophytic bacteria. *Biolog Cont* 5, 83–91.
- Chet, I., Ordentlich, A., Shapira, R., Oppenheim, A., 1990. Mechanisms of biocontrol of soilborne plant pathogens by rhizobacteria. *Plant Soil* 129, 85–92.
- Idris, H.A., Labuschagne, N., Korsten, L., 2007. Screening rhizobacteria for biological control of *Fusarium* root and crown rot of sorghum in Ethiopia. *Biolog Cont* 40 (1), 97–106.
- Kai, M., Hausteine, M., Molina, F., Petri, A., Scholz, B., Piechulla, B., 2009. Bacterial volatiles and their action potential. *Appl Microbiol Biotechnol* 81 (6), 1001–1012.
- Kobayashi, D.Y., Reedy, R.M., Bick, J., Oudemans, P.V., 2002. Characterization of a chitinase gene from *Stenotrophomonas maltophilia* strain 34S1 and its involvement in biological control. *Appl Environ Microbiol* 68 (3), 1047–1054.
- Raaijmakers, J.M., Bonsall, R.F., Weller, D.M., 1999. Effect of population density of *Pseudomonas fluorescens* on production of 2, 4-diacetylphloroglucinol in the rhizosphere of wheat. *Phytopathology* 89 (6), 470–475.
- Singh, V.K., Jayaswal, R.K., Wilkinson, B.J., 2001. Cell wall-active antibiotic induced proteins of *Staphylococcus aureus* identified using a proteomic approach. *FEMS Microbiol Lett* 199 (1), 79–84.
- Sonawane, A., Klöppner, U., Hövel, S., Völker, U., Röhm, K.H., 2003. Identification of *Pseudomonas* proteins coordinately induced by acidic amino acids and their amides: a two-dimensional electrophoresis study. *Microbiology* 149 (10), 2909–2918.
- Stein, T., 2005. *Bacillus subtilis* antibiotics: structures, syntheses and specific functions. *Mol Microbiol* 56 (4), 845–857.
- Yaoyao, E., Yuan, J., Yang, F., Wang, L., Ma, J., Li, J., Pu, X., Raza, W., Huang, Q., Shen, Q., 2017. PGPR strain *Paenibacillus polymyxa* SQR-21 potentially benefits watermelon growth by re-shaping root protein expression. *AMB Expr* 7, 104–115.
- Zhender, G.W., Yao, C., Murphy, J.F., Sikora, E.R., Kloepper, J.W., 2000. Induction of resistance in tomato against cucumber mosaic cucumovirus by plant growth-promoting rhizobacteria. *Bio Control*, 45 (1), 127–137.



Amelioration of Salinity Stress by PGPR: ACC Deaminase and ROS Scavenging Enzymes Activity

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5.1 INTRODUCTION

The UN projections suggest that the world population possibly will reach 9.15 billion in 2050 (www.fao.org/economic/esa). This incessantly expanding global population raises a crucial demand for prevailing agricultural systems to initiate resolution to a progressively ever-increasing demand in food production. Soil salinity is one of the primary abiotic stresses accountable for loss of plant growth, crop yield, and productivity. More than 800 million hectares of land (6% of the total land mass) are potentially utilizable for agriculture but are afflicted with salt stress across the globe and hence relegate agricultural production (Munns and Tester, 2008). Food and Agricultural Organization (FAO), has predicted that by the year 2050, the expanse of 50% of total land mass will be lost due to salinity (Munns, 2002; Ilangumaran and Smith, 2017).

Plants subjected to excess salt initiates ionic imbalance, which leads to metabolism imbalances induced by ion toxicity and water deficit generated by hyperosmotic stress. The surge in reactive oxygen species (ROS) owing to saline environments triggers cellular toxicity in plants. Hence, the responsibility of the antioxidant system in shielding plants against salinity induced cellular stress is considerably significant. Root systems are crucial in convalescing crop salt tolerance via their capability for enhancing availability of water and nutrients and restraining salt accumulation (Jung and McCouch, 2013).

Large numbers of methods have been developed to lessen the grave results of salinity pressure on plants. Numerous reports have attended this question by developing genetic engineering options (Xu et al., 2015; Liang et al., 2018), nevertheless these techniques are arduous and with the poor acceptance of genetically modified crops by governments across the globe owing to their unpredicted environmental perils, the genetically modified/engineered plants are seldom grown in the field. Plant growth promoting rhizobacteria (PGPR) are microbes which inhabit the plant roots and enhance plant development by direct or indirect methods. Numerous researches are available that elucidate the influence of PGPR in alleviating abiotic stress in diverse crop plants (Sharma et al., 2017). Besides their growth-promoting ability, a few PGPR are also acknowledged to reduce effects of salinity stress and hence, the employment of PGPR for improved salt tolerance has substantial benefits in comparison to other methods. Several PGPR have been explored and utilized as valuable and competent drivers for promoting salt tolerance in plants owing to their capacity to elevate plant growth and to tenaciously inhabit plant roots. In recent times, it has been demonstrated that various PGPR genera, such as *Pseudomonas*, *Azospirillum*, *Burkholderia*, *Arthrobacter*, *Bacillus*, *Enterobacter*, and *Azotobacter* can improve salt tolerance in several agricultural crops. For example, inoculating *Bacillus* sp. in plants, for example, *Arabidopsis thaliana*, wheat, maize, and rice, can check inadequate growth and enhance the performance of plants in adverse conditions. in saline conditions. The communication among plants and PGPR is a multifaceted and mutual process.



5.2 SALINITY STRESS AND ROS

ROS production in plants is of a general occurrence owing to innumerable metabolic responses that ensue in various locations in a plant cell. However, ROS levels upsurge significantly, resulting in redox imbalance and oxidative stress in plants exposed to abiotic stresses, for example, salinity, drought, extreme heat and cold, and metal or metalloid stresses (Choudhury et al., 2017). Abiotic stresses check the CO₂ fixation and lessen the production of NADP⁺ during Calvin cycle. Consequently, decline in the photosynthetic electron transport chain (ETC) ensues

which produces superoxide radicals and singlet oxygen in the chloroplasts (Gill and Tuteja, 2010). Studies have suggested that photorespiration is credited for approximate 70% or more of the H_2O_2 generated under osmotic/salt stress (Noctor et al., 2002).



5.3 ROS SCAVENGING IN PLANTS

Plants possess enzymatic and nonenzymatic antioxidant defense mechanisms which curb the generation of the extremely toxic ROS, and help in foraging ROS and leading to resistance to oxidative damage in plant cells (Foyer and Noctor, 2005). Plants have developed inherent machinery to perceive, transduce, and translate ROS signals into suitable cellular communications. This route entails the presence of redox-sensitive proteins that can participate together in oxidation and reduction reactions and might synchronize the switching-on or -off liable to the cellular redox state (Shao et al., 2008). Such redox-sensitive proteins are oxidized by ROS via the omnipresent redox-sensitive entities [thioredoxins (Trxs) or glutathione] directly or indirectly (Nakashima and Yamaguchi-Shinozaki, 2006). Primarily, this encompasses relegating the intracellular water deficit via amplified osmotic adjustments, and eliminating excessive ROS through an antioxidant defense system comprising of enzymes that comprehend and neutralize stress (Gill and Tuteja, 2010; Porcel et al., 2003).

Salinity stress leads to the formation of ROS, namely, superoxide (O_2^-), singlet oxygen (O_2), hydroxyl (OH^-), and hydrogen peroxide (H_2O_2), which cause severe damage to cell structures by exerting oxidation of cell membranes in a process known as oxidative stress. However, a defense mechanism called the antioxidant enzyme system is also triggered during stress conditions.

The antioxidant systems that influence H_2O_2 levels involve of both non-enzymatic and enzymatic H_2O_2 scavengers. Enzymes, such as catalase (CAT), ascorbate peroxidase (APX), glutathione peroxidase (GPX), glutathione *S*-transferases (GSTs), glutathione reductase (GR), and peroxiredoxin (Prx), and nonenzymatic compounds, such as ascorbate (AsA), glutathione (GSH), α -tocopherol and flavonoids, are regularly engaged in controlling the ROS quantities, including H_2O_2 (Miller et al., 2010; Kapoor et al., 2015).

Phytohormones, such as IAA and ethylene, play a crucial role in signal transduction pathways activated during salt stress as plant responses to saline conditions (Wani et al., 2016).

In addition to excessive ROS production, salt stress leads to severe dehydration in plant cells. Proline is among the most important osmoprotectants in plant cells that is rapidly accumulated under salt stress. To a certain extent, proline may maintain an osmotic pressure balance between intracellular and extracellular space to improve the overall moisture-holding capacity, and reduce the damage caused by salt stress (Lei et al., 2016).

In addition to ROS-scavenging enzymatic and nonenzymatic antioxidants, over-expressing ROS-responsive signaling and regulatory genes are also accountable for stress tolerance in plants. The regulatory genes regulating a large set of genes participating in salt mitigating mechanisms, including ROS-scavenging enzymes proved beneficial in improved tolerance. In *Arabidopsis*, over-expression of mitogen-activated kinase kinase 1 (MKK1) improved the MAPK cascade activity, which is also activated by ROS (Smékalová et al., 2014) leading to enhanced tolerance to abiotic stress by monitoring stress-associated ROS concentrations under abiotic stress (Miller et al., 2010). Similarly, over-expression of transcription factors (*Zat12* or *JERF3*, *Zat10*) control the expression of various ROS-scavenging genes encoding enzymes which in turn leads to higher tolerance to salt/osmotic stress (Nachimuthu et al., 2017).



5.4 ETHYLENE IN SALINITY STRESS

Ethylene is a gaseous hormone which fulfills manifold responsibilities in managing and regulating growth and development of plants and also operates or modulates plant response to environmental stresses and participates in systematic growth (Schaller and Voesenek, 2015). When the content of ethylene in plant tissues has been analyzed, two levels of ethylene peak have been observed. The initial peak is always smaller and appears at an early phase of stress exposure to the plant and it acts as a signal to turn on the protective defense responses. Whereas second peak is much larger than the first one, and appears after few hours to few days of stress application. This level of stress ethylene is responsible for inhibitory

effects of plant survival and causes chlorosis of leaves, cell damage, abscission, senescence, and death. (Stearns and Glick, 2003). ACC is a precursor for ethylene synthesis and during onset of stress conditions the ACC level in plant tissues is also very low. Here ACC oxidase (ACO) enzyme plays its role and it is induced to convert the major amount of ACC into ethylene. ACO autocatalyzes its own synthesis and is also responsible for turning on ACC synthase (ACS) expression during stress (Kende, 1993; Fluhr et al., 1996). Once the pool of ACC has been consumed there is a lag in ethylene production until more ACC can be synthesized by the action of ACS. The genes for ACS are regulated by environmental and developmental cues (Tsuchisaka and Theologis, 2004) and its enzymatic action is enhanced during stress conditions (Yang and Hoffman, 1984). There are enzymes which can degrade (S'-adenosyl-L-methionine) SAM or (1-aminocyclopropane-1-carboxylic acid) ACC (precursor of ethylene). Some ethylene inhibitors can effectively reduce the levels of ethylene without significantly altering plant physiology (Klee, 1993; Robison et al., 2001; Iqbal et al., 2013) and lowering of ethylene levels or perception have conferred salt stress in many plants (Mayak et al., 2004b; Barnawal et al., 2014; Barnawal et al., 2017).

5.4.1 Regulation of plant stress ethylene levels

Phytohormones play vital role in plant developmental and stress signaling processes (Nascimento et al., 2014). Ethylene is considered as stress plant hormone since very long (Morgan and Drew, 1997). So, modulation in stress ethylene levels affects its signaling during stress responses. As ethylene signaling is important for plant growth and stress regulations, the severity of stress lowers as its concentration decreases in plant tissues. Its level can be maintained either by developing transgenic plants or involving ACC deaminase-containing microbes which can regulate stress ethylene levels up to certain extent so that plants get protected and become healthier under stress.

Ethylene is perceived by an ethylene receptor related to bacterial two component regulators. Ethylene binding leads to inactivation of the receptors and subsequently, downstream protein Constitutive Triple Response1 (CTR1) is inactivated. CTR1 inactivation led to activation of the downstream protein Ethylene Insensitive2 (EIN2), a positive component of ethylene signaling pathway and consequent activation of transcription factors, EIN3 and Ethylene Response Factor (ERF) trigger

innumerable genes and is known as the ethylene responsive gene (Yasumura et al., 2015).

Ethylene plays a role in the regulation and maintenance of many physiological responses (Arshad et al., 2002). Ethylene, participates in an extensive range of growth and development pathways like seed germination, root hair development, root elongation, leaf and petal abscission, fruit ripening and organ senescence (Tao et al., 2015), but it is promptly synthesized in plants when exposed to biotic and abiotic stresses and is discerned to stimulate the expression of numerous stress-associated genes (O'donnell et al., 2001). This emission of ethylene owing to its negative impact on the plant under duress is usually termed as “stress ethylene” (Saleem et al., 2007). Decreasing the concentration of ethylene in the plant reduces the degree of ethylene inhibited root elongation and plants growth (Bleecker and Kende, 2000; Forni et al., 2017). Production of ethylene in plants is directly correlated with endogenous ACC (1-amino-cyclopropane-1-carboxylic acid) levels (Shaharouna et al., 2006). Under nonstressed conditions, ethylene helps in root elongation, root hair development, fruit ripening and organ senescence (Giovannoni, 2001). But as stress response, production of high level of stress ethylene occurs and acts antagonistically for normal function and damaging to plant growth. In *Tamarix hispida*, ERF1 impedes the expression of SOD and POD genes in drought or high salinity, consequently resulting in higher ROS concentrations due to diminished scavenging capacity (Wang et al., 2014).



5.5 PLANT GROWTH PROMOTING RHIZOBACTERIA

There are certain group of bacteria which provide beneficial effects on total plant growth, development, and yield, these bacteria are termed as PGPR (Kloepper and Schroth, 1978). Since these bacteria are recognized as important for improved emergence of seedlings, plant biomass, and better root system proliferation, a lot of work has been already done on identifying plant growth promoting bacteria present in natural ecosystems and development of some bacterial strains for commercial use (Kishore et al., 2006). PGPR must have some distinctive characters: (1) they should be capable enough to colonize the root surface; (2) they should compete, multiply and survive with other microbiota; and (3) they must promote plant growth

(Lugtenberg and Kamilova, 2009). It is estimated that about 2%–5% of rhizobacteria are plant growth promotive in nature (Kloepper and Schroth, 1981). So, several soil bacterial species enhance plant growth by a plethora of mechanisms and grow in, on or plant root tissues called as PGPR.

Plant growth promotion by bacteria can be followed by direct and indirect mechanisms. Direct mechanisms involve nitrogen fixers (Pii et al., 2015), phyto-stimulators (plant growth promotion by hormone production), rhizo-remediators (decomposing organic pollutants), and biopesticides (chiefly by producing various antibiotics and antifungal metabolites) (Ahemad and Kibret 2014), metabolism of the ethylene precursor 1-aminocyclopropane-1-carboxylate (ACC) by the action of enzyme ACC deaminase (Gamalero and Glick, 2015) and improved accessibility of iron via siderophores produced by various kind of bacteria (Ahemad and Kibret, 2014).

Regarding the consequences of soil salinity on crop growth and productivity, striving to alleviate the harmful effects of salinity on plant growth and development via PGPR inoculation can be of significance. Microbes mediated amelioration of plant stress has developed as a valuable segment of salt stress management in plants and various studies have established their importance in improving growth and productivity considering sustainable agriculture (Venkateswarlu et al., 2008; Grover et al., 2011; Coleman-Derr and Tringe, 2014).

5.5.1 ACC deaminase-containing bacteria

Plant growth promoting bacteria containing 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity promote plant growth. ACC is an immediate precursor of ethylene in all higher plants. ACC deaminase is studied as multimeric enzyme that cleaves ACC to α -ketobutyrate and ammonia and therefore, decreases ethylene levels of the host plant (Glick, 2014). PGPR often interfere with the biosynthesis of phytohormones, especially auxin and ethylene.

ACC is the immediate precursor of ethylene in higher plants (Yang and Hoffman, 1984) and its regulation has been described as the principal mechanism by which bacteria exert beneficial effects on plants under abiotic stress (Saleem et al., 2007). Certain microorganisms have been reported to contain the enzyme ACC deaminase that hydrolyzes ACC into ammonia and α -ketobutyrate (Glick et al., 2007) instead of converting it into ethylene. The cleavage of ACC by ACC deaminase-containing

rhizobacteria reduces the ACC and ethylene levels in the rhizoplane, thereby providing a sink for the ACC. These reduced ACC levels in turn decrease the levels of endogenous ethylene, thus eliminating the potentially inhibitory effects of higher ethylene concentrations.

ACC deaminase is normally present in bacteria in comparatively low concentrations until it is induced, and the induction of enzyme activity is a relatively gradual and intricate process. ACC deaminase lowers the stress ethylene levels by sequestering ACC content of the plant tissues and promotes plant growth under stress situations (Glick 2005; Argueso et al., 2007). A model of these bacterial functions has been published which describes the role of bacterial ACC deaminase in decreasing the level of stress ethylene. It alters plant physiology under stressed and nonstressed conditions (Glick et al., 2007).

5.5.2 PGPR and ROS scavenging in salt stress

Various mechanisms that PGPR employ to shield plants from salt stress are intricately linked and modulate one another. However, a comprehensive explanation of the attributes of these interrelated mechanisms remains, yet to be elucidated, mostly. Mitigation of oxidative impairment by antioxidant enzymes mediated ROS scavenging is a crucial approach in plants to increase tolerance to salinity stress.

The endurance of the bacterial strains in the highly competitive rhizosphere is credited to their role in the plant growth promotion as well as mitigation of abiotic stress (Dimkpa et al., 2009). Bacteria moderate the redox state of salt-affected plants by boosting antioxidants and polyamines, which results in amplified photosynthetic efficiency (Radhakrishnan and Baek, 2017). PGPR generate antioxidants such as catalase and neutralize ROS-mediated oxidative stress. Improved activities of SOD in PGPR-inoculated salt-stressed plants can perform a vital part in rummaging superoxide radicals.

Improvement of plant-microbe interaction in salinity affected areas can be understood by osmoadaptation mechanism of *Azospirillum sp.* because nitrogenase synthesis and activity is affected by salt stress (Tripathi et al., 2002) so it accumulates compatible solutes such as glutamate, glycinebetaine, proline, and trehalose under salinity stress conditions which play major role in osmoadaptation. It shifts dominant level of osmolyte from glutamate to proline when inoculated to sorghum plants and with higher volume of water content and greater water potential and lesser

canopy temperature in comparison to noninoculated stressed plants (Tripathi et al., 1998).

Inoculation of potato plants with two *Bacillus* strains belonging not only induced substantial variations in the gene expression of diverse ROS-scavenging enzymes but also improved the photosynthetic efficiency of bacteria-inoculated plants, which could be attributed as a significant explanation for the enhanced abiotic stress tolerance (Gururani et al., 2013). *Enterobacter cloacae* HSNJ4 alleviated the oxidative damage in canola seedlings caused by ROS produced owing to salt stress HSNJ4 enhanced SOD, POD, and CAT activity in canola seedlings, ROS scavenging capacity under salt stress increased, thereby reducing the MDA content. HSNJ4 inoculation contributed to maintaining the activity of these three antioxidant enzymes at a higher level compared with the control groups under salt stress conditions (Li et al., 2017).

To recognize *Bacillus amyloliquefaciens* FZB42 inoculated plant genes with significant roles in salt stress mitigation, the transcriptome profiles of salinity affected *Arabidopsis* shoot tissues was investigated via Illumina sequencing. The RNA-seq data exhibited that FZB42 stimulated the upregulation of genes associated with photosynthesis, ROS scavenging, osmoprotectants such as trehalose and proline, Na^+ translocation as well as jasmonic acid, auxin, and ethylene signaling in salt stress conditions (Liu et al., 2017). *Dietzia natronolimnaea* STR1-inoculated salt-stressed wheat plants documented augmented TaWRKY10 expression. WRKY TFs take part in the significant roles in regulation stress by moderating the cellular osmotic balance, ROS scavenging mechanism and expression of different stress-related genes (Bharti et al., 2016). Inoculation of rice (*Oryza sativa*) with two root-associated bacteria *Pseudomonas pseudoalcaligenes* and *Bacillus pumilus* could impart salt tolerance by relegating the toxic reactive oxygen species by lessening plant cell membrane index, cell caspase like protease activity, and programed cell death and hence lead to improved cell viability. In this study, the PGPR inoculated paddy indicated reduction in SOD and APX activities signifying a reduced O_2^- scavenging and dismutating capacity in the GJ17 cultivar and indicates a probable participation of the two enzymes in salt tolerance (Jha et al., 2011).

The variance expression of antioxidant genes may possibly be implicated in the regulation of ROS level in PGPR inoculated plants under salinity stress. This corresponds with earlier studies where PGPR treatment stimulated antioxidant defense mechanism, in so doing resulting in decreased concentration of ROS in plants exposed to salt stress (Heidari

and Golpayegani, 2012; Upadhyay et al., 2012; Gururani et al., 2013). Salt stressed (75 and 150 mM) maize plants inoculated with *Staphylococcus sciuri* SAT-17 demonstrated considerable reduction in ROS levels and improved antioxidant enzymes (CAT, POD) levels, implying that the PGPR can stimulate plant's inherent mechanism to combat oxidative stress when induced under salinity stress (Akram et al., 2016). The red pepper plants inoculated with ACC deaminase-containing *Pseudomonas frederiksbergensis* OS261 showed a significant elevation of the antioxidant enzyme CAT compared to the un-inoculated plants indicating that the plants adapted to salinity stress by reducing ROS (Chatterjee et al., 2017). In the *Pseudomonas frederiksbergensis* OS261 inoculated plants, the SOD and APX activities in higher salinity levels exhibited a substantial decrease hinting the neutralization of harmful ROS entities resulting in mitigation of salt stress. The probable cause of the reduction could be the shortage of hydrogen peroxide generation in the plants inoculated with bacteria, where PGPR played a major role in sustaining the homeostasis of plant system (Gururani et al., 2013). The antioxidant enzyme activities have been researched considerably, but still the implication of enzymes in salt tolerance is unclear, as increased antioxidant activities are associated with both salinity tolerance and sensitivity (Abogadallah, 2010).

Burkholderia phytofirmans PsJN treatment in *Arabidopsis* augmented the accretion of proline and transcription of genes associated with abscisic acid signaling (*Relative to Dessication*, *RD29A*, and *RD29B*), ROS scavenging (*Ascorbate Peroxidase 2*), and detoxification (*Glyoxalase I 7*), and down-regulated the expression of *Lipoxygenase 2* (related to jasmonic acid biosynthesis) (Pinedo et al., 2015). Table 5.1 illustrates ROS-mediated amelioration of salinity stress in various PGPR inoculated plants.

5.5.3 ACC deaminase containing rhizobacteria in salt stress

Salt stress leads to discrepancy in ethylene generation, as a rise in ethylene level can impede root and shoot length and thus consequently reduction in an overall growth and development of plants (Nadeem et al., 2010). Earlier studies have documented that bacteria having ACC deaminase activity reduce the level of stress ethylene conferring stress resistance and growth of plant under salt stress.

The ACC deaminase-containing PGPR are usually present in various kinds of stressed soils affected by floods, salinity, drought, and phytopathogens and promote plant growth and yield by employing key mechanisms mainly through sinking ethylene concentrations by

Table 5.1 Amelioration of salt stress by PGPR via modulation of ROS

Bacteria	Mechanisms	Plant	References
<i>Pseudomonas</i>	Induction of antioxidant enzymes	<i>Lettuce</i>	Kohler et al. (2009)
<i>Pseudomonas pseudoalcaligenes</i> , <i>Bacillus pumilus</i>	Increased concentration of glycine betaine (compatible solute) Decrease in APX, Increase in nitrate reductase activity	Rice	Jha et al. (2011)
<i>Bacillus spp.</i>	Increased accumulation of proline, sugars, free amino acids and decrease electrolyte leakage. Reduced activity of antioxidants enzyme (catalase, glutathione peroxidase)	<i>Zea mays</i>	Vardharajula et al. (2011)
<i>Bacillus subtilis</i> SU47 and <i>Arthrobacter sp.</i>	Increase in total soluble sugars and proline content	<i>Wheat</i>	Upadhyay et al. (2012)
<i>Azospirillum</i>	Increased ascorbic acid content	<i>Lettuce</i>	Fasciglione et al. (2012)
<i>Bacillus amyloliquefaciens</i>	Differential transcription of genes involved in antioxidant machinery	Rice	Nautiyal et al. (2013)
<i>Azospirillum lipoferum</i>	Increase accumulation of soluble sugar, free amino acids and proline	Maize (<i>Zea mays</i>)	Bano et al. (2013)
<i>Exiguobacterium oxidotolerans</i>	Improved proline content, antioxidant enzyme activity	<i>Bacopa monnieri</i>	Bharti et al. (2013)
<i>Exiguobacterium oxidotolerans</i>	Phosphate nutrition, Proline, K ⁺ uptake, Increased CAT, APX activity	<i>Mentha</i>	Bharti et al. (2014)
<i>Bacillus spp.</i>	Increased activity of SOD, CAT, phenols, increased proline	<i>Gladiolus</i>	Damodaran et al. (2014)
<i>Enterobacter spp.</i>	IAA, Increased expression of salt stress responsive genes such as DREB2b, RD29A, RD29B,	Tomato	Kim et al. (2014)

(Continued)

Table 5.1 (Continued)

Bacteria	Mechanisms	Plant	References
<i>Burkholderia cepacia</i> , <i>Promicromonospora</i> <i>spp.</i> , <i>Acinetobacter</i> <i>calcoaceticus</i>	RAB18 in Arabidopsis, Higher APX activities Reduced activities of CAT, POD, polyphenol peroxidase (PPO), increased gibberellins	Cucumber	Kang et al. (2014)
<i>Azotobacter vinelandii</i>	Higher proline and malondialdehyde content, indole-3 acetic acid (IAA), gibberellins (GA3), zeatint (Zt)	Rice	Sahoo et al. (2014)
<i>Enterobacter spp.</i>	Enhanced amino acids, suppressed salicylic acid synthesis	Cucumber	Kang et al. (2015)
<i>Burkholderia</i> <i>phytofirmans</i> PsJN	ABA-dependent pathways; ROS scavenging and detoxifying	<i>Arabidopsis</i>	Pinedo et al. (2015)
<i>Enterobacter sp.</i> <i>UPMR18 (ACC</i> <i>deaminase)</i>	Increase antioxidant enzyme activities and upregulation of ROS pathway genes	<i>Abelmoschus</i> <i>esculentus</i>	Habib et al. (2016)
<i>Pseudomonas putida</i> <i>MTCC5279 (RA)</i>	Osmolyte accumulation, ROS scavenging ability and stress-responsive gene expressions	<i>Cicer</i> <i>arietinum</i> <i>L.</i>	Tiwari et al. (2016)
<i>Dietzia natronolimnaea</i>	Induction of <i>TaMYB</i> and <i>TaWRKY</i> expression, enhanced antioxidant enzymes	wheat	Bharti et al. (2016)
<i>Pseudomonas</i> <i>frederiksbergensis</i> <i>OS261</i>	Increased CAT and reduced SOD and APX	Red pepper	Chatterjee et al. (2017)
<i>Bacillus megaterium</i>	Adjustment of JA metabolism	<i>Arabidopsis</i>	Erice et al. (2017)

1-aminocyclopropane-1-carboxylic acid (ACC) deamination as ethylene is the subsequent product generated from ACC (Glick et al., 2007).

PGPR which contain the enzyme ACC deaminase, operate as a sink for ACC, the immediate biosynthetic precursor of ethylene, thus reducing plant ethylene levels (Glick, 2005). ACC deaminase-containing bacterial treated seedlings produced reduced quantity of ethylene with respect to

control plants (minus microbial inoculations) exposed to salt stress (Barnawal et al., 2017). Thus, ACC deaminase-containing bacteria limit the production of ethylene in plants exposed to salt stress conditions, which may possibly be an effective method in mitigating some percentage of the stress effect.

The enzyme ACS is involved in ACC biosynthesis. In ethylene biosynthesis S-adenosyl-L-methionine (SAM) is converted to ACC and then to ethylene via ACS and ACO enzymes, respectively. The activity of ACS amplified as the level of salt stress enhanced irrespective of microbiological treatments.

Salinity stress affects crop productivity in a negative manner, an attempt of mitigating stress by utilizing ACC deaminase-containing plant growth promoting microbes can be of remarkable importance. The following cases exemplify the role of ACC deaminase producing PGPR in inducing and managing salt tolerance in the inoculated plants. The inoculation of plant growth promoting bacteria alleviates plant stress. For example, soil salinity is one of the most severe growth limiting environmental factor which limits nodulation, biomass, and physiological responses of leguminous crops (Han and Lee, 2005).

Klebsiella sp. SBP-8 containing ACC deaminase activity significantly increased the salinity tolerance of wheat (Singh et al., 2015). Likewise, under salt stress, inoculation with *Pseudomonas putida* UW4 having ACC deaminase activity significantly enhanced a suite of growth parameters in canola (Li et al., 2017). Application of *Enterobacter cloacae* HSNJ4 significantly decreased the stress from ethylene and increased the salt tolerance of plants. When ACC was exuded through the root of canola, it was degraded by HSNJ4 to produce α -ketobutyrate and ammonia, thereby decreasing the level of ethylene (Ali et al., 2014). Three PGPR strains *P. fluorescens*, *P. aeruginosa*, and *P. stutzeri* were isolated from the tomato rhizosphere encompassing higher concentration of sodium chloride. These microbes could stimulate production of phytohormones and ACC deaminase enzyme to convalesce salinity tolerance in tomato plant (Bal et al., 2013; Tank and Saraf, 2010). *Achromobacter piechaudii* ARV8 producing ACCD enhanced tolerance in peppers and tomato to drought and salt stresses (Mayak et al., 2004a, b). *Pseudomonas putida* UW4 inoculated tomato (*Solanum lycopersicum*) seedlings displayed improved shoot growth after 6 weeks in saline conditions up to 90 mM NaCl. An upregulation in the expression pattern of *Toc GTPase*, a gene of the chloroplast protein import apparatus was observed, which might help import of proteins

involved as a mode of stress response (Yan et al., 2014). Table 5.2 shows the role of ACC deaminase containing bacteria in ameliorating salinity stress in a wide variety of crops.

Table 5.2 Amelioration of salt stress by ACC-deaminase containing PGPR

Plants	Bacteria	Reference
Wheat	<i>Arthrobacter protophormiae</i>	Barnawal et al. (2017)
Chinese cabbage	<i>Herbaspirillum</i> sp.	Lee et al. (2016)
Cucumber	<i>P. fluorescens</i>	Egamberdieva et al. (2011); Cho et al. (2015)
Cotton	<i>Klebsiella oxytoca</i>	Yue et al. (2007); Liu et al. (2013)
Barley	<i>Hartmannibacter diazotrophicus</i>	Suarez et al. (2015)
Rice	<i>P. stutzeri</i>	Han et al. (2015)
Pea	<i>Arthrobacter protophormiae</i>	Barnawal et al. (2014)
Maize	<i>P. syringae</i> , <i>P. fluorescens</i>	Zafar-ul-Hye et al. (2014)
Barley, oats	<i>P. putida</i> , <i>P. sp.</i> , <i>P. corrugata</i>	Chang et al. (2014)
<i>Limonium sinense</i> (Girard)	<i>Bacillus</i> , <i>Arthrobacter</i> , <i>Streptomyces</i> , <i>Isoptricola</i>	Qin et al. (2014)
Kuntze		
Tomato,	<i>Enterobacter</i> sp.	Kim et al. (2014)
Arabidopsis		
Chickpea	<i>Mesorhizobium ciceri</i>	Brígido et al. (2013)
Mung bean,	<i>Bradyrhizobium</i> sp., <i>Enterobacter</i> sp.,	Tittabutr et al. (2013)
bean, peanut	<i>Chryseobacterium</i> sp.	
Mung bean	<i>P. syringae</i> , <i>P. fluorescens</i>	Ahmad et al. (2013)
Wheat	<i>Serratia</i> spp., <i>Aerococcus</i> spp.	Bangash et al. (2013)
Mung bean	<i>Rhizobium</i> sp.	Aamir et al. (2013)
Red pepper	<i>Brevibacterium iodinum</i> , <i>Bacillus licheniformis</i> , <i>Zhihengliuella alba</i>	Siddikee et al. (2011)
Barley	<i>Paenibacillus polymyxa</i> , <i>Bacillus megaterium</i> , <i>Bacillus cereus</i> , <i>Bacillus pumilus</i>	Timmusk et al. (2011)
Tomato	<i>P. mendocina</i>	Sadrnia et al. (2011)
Cucumber	<i>P. putida</i>	Gamalero et al. (2010)
Maize	<i>Pseudomonas fluorescens</i>	Nadeem et al. (2010)
<i>Arachis hypogea</i>	<i>Pseudomonas fluorescens</i>	Saravanakumar and Samiyappan (2007)
Canola	<i>P. putida</i>	Cheng et al. (2007)
Tomato	<i>Achromobacter piechaudii</i>	Mayak et al. (2004b)



5.6 FUTURE PROSPECTIVE

Salinity is posing serious threat to agriculture and the environment. And as the saline areas under agriculture are expanding annually across the globe, making it a matter of concern for agriculturists and policy makers. It is not only suppressing the plant growth but also disturbing the sustainability of beneficial microorganisms associated with the plant rhizosphere.

The knowledge concerning the molecular signals and pathways driving the beneficial plant-microbe interactions is still limited; and lesser is known regarding the relationship between phytohormones in PGPR inoculated plants and their cumulative response to salinity stress at the whole plant scale. The systems biology approach for unraveling and understanding the intricacies of plant-microbe interactions under salt stressed conditions offers novel possibilities for employing the rhizosphere bacteria as sustainable agents of crop improvement.

Research focused toward the utilization of PGPR in salt-affected agricultural fields promotes development of bacterial inoculants as commercial biofertilizers for improved salinity tolerance.

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REFERENCES

- Aamir, M., Aslam, A., Khan, M.Y., Usman, M., 2013. Co-inoculation with *Rhizobium* and plant growth promoting rhizobacteria (PGPR) for inducing salinity tolerance in mung bean under field condition of semi-arid climate. *Asian J Agri Biol* 1 (1), 7.
- Abogadallah, G.M., 2010. Insights into the significance of antioxidative defence under salt stress. *Plant Signal Behav* 5 (4), 369–374.
- Ahemad, M., Kibret, M., 2014. Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. *J King Saud Univ Sci* 26 (1), 1–20.
- Ahmad, M., Zahir, Z.A., Khalid, M., Nazli, F., Arshad, M., 2013. Efficacy of *Rhizobium* and *Pseudomonas* strains to improve physiology, ionic balance and quality of mung bean under salt-affected conditions on farmer's fields. *Plant Physiol Biochem* 63, 170–176.
- Akram, M.S., Shahid, M., Tariq, M., Azeem, M., Javed, M.T., Saleem, S., et al., 2016. Deciphering *Staphylococcus sciuri* SAT-17 mediated anti-oxidative defense mechanisms and growth modulations in salt stressed maize (*Zea mays* L.). *Front Microbiol* 7, 867.

- Ali, S., Charles, T.C., Glick, B.R., 2014. Amelioration of high salinity stress damage by plant growth-promoting bacterial endophytes that contain ACC deaminase. *Plant Physiol Biochem* 80, 160–167.
- Argueso, C.T., Hansen, M., Kieber, J.J., 2007. Regulation of ethylene biosynthesis. *J Plant Growth Regul* 26 (2), 92–105.
- Arshad, M., Frankenberger Jr, W.T., 2002. *Ethylene: Agricultural Sources and Applications*. Kluwer Academic Publishers, New York, NY, p. 342.
- Bal, H.B., Nayak, L., Das, S., Adhya, T.K., 2013. Isolation of ACC deaminase producing PGPR from rice rhizosphere and evaluating their plant growth promoting activity under salt stress. *Plant Soil* 366 (1–2), 93–105.
- Bangash, N., Khalid, A., Mahmood, T., Siddique, M.T., 2013. Screening rhizobacteria containing ACC-deaminase for growth promotion of wheat under water stress. *Pakistan J Bot* 45, 91–96.
- Bano, Q., Ilyas, N., Bano, A., Zafar, N., Akram, A., Hassan, F., 2013. Effect of *Azospirillum* inoculation on maize (*Zea mays* L.) under drought stress. *Pak. J. Bot.* 45 (S1), 13–20.
- Barnawal, D., Bharti, N., Maji, D., Chanotiya, C.S., Kalra, A., 2014. ACC deaminase-containing *Arthrobacter protophormiae* induces NaCl stress tolerance through reduced ACC oxidase activity and ethylene production resulting in improved nodulation and mycorrhization in *Pisum sativum*. *J Plant Physiol* 171 (11), 884–894.
- Barnawal, D., Bharti, N., Pandey, S.S., Pandey, A., Chanotiya, C.S., Kalra, A., 2017. Plant growth promoting rhizobacteria enhances wheat salt and drought stress tolerance by altering endogenous phytohormone levels and TaCTR1/TaDREB2 expression. *Physiol. Plant.* 161 (4), 502–514.
- Bharti, N., Barnawal, D., Awasthi, A., Yadav, A., Kalra, A., 2014. Plant growth promoting rhizobacteria alleviate salinity induced negative effects on growth, oil content and physiological status in *Mentha arvensis*. *Acta Physiol Plan* 36 (1), 45–60.
- Bharti, N., Pandey, S.S., Barnawal, D., Patel, V.K., Kalra, A., 2016. Plant growth promoting rhizobacteria *Dietzia natronolimnaea* modulates the expression of stress responsive genes providing protection of wheat from salinity stress. *Sci Rep* 6.
- Bharti, N., Yadav, D., Barnawal, D., Maji, D., Kalra, A., 2013. Exiguobacterium oxidotolerans, a halotolerant plant growth promoting rhizobacteria, improves yield and content of secondary metabolites in *Bacopa monnieri* (L.) Pennell under primary and secondary salt stress. *World J Microbiol Biotechnol* 29 (2), 379–387.
- Bleecker, A.B., Kende, H., 2000. Ethylene: a gaseous signal molecule in plants. *Annu Rev Cell Dev Biol* 16 (1), 1–18.
- Brígido, C., Nascimento, F.X., Duan, J., Glick, B.R., Oliveira, S., 2013. Expression of an exogenous 1-aminocyclopropane-1-carboxylate deaminase gene in *Mesorhizobium* spp. reduces the negative effects of salt stress in chickpea. *FEMS Microbiol Lett* 349 (1), 46–53.
- Chang, P., Gerhardt, K.E., Huang, X.D., Yu, X.M., Glick, B.R., Gerwing, P.D., et al., 2014. Plant growth-promoting bacteria facilitate the growth of barley and oats in salt-impacted soil: implications for phytoremediation of saline soils. *Int J Phytoremediation* 16 (11), 1133–1147.
- Chatterjee, P., Samaddar, S., Anandham, R., Kang, Y., Kim, K., Selvakumar, G., et al., 2017. Beneficial soil bacterium *Pseudomonas frederiksbergensis* OS261 augments salt tolerance and promotes red pepper plant growth. *Front Plant Sci* 8, 705.
- Cheng, Z., Park, E., Glick, B.R., 2007. 1-Aminocyclopropane-1-carboxylate deaminase from *Pseudomonas putida* UW4 facilitates the growth of canola in the presence of salt. *Can J Microbiol* 53 (7), 912–918.
- Cho, S.T., Chang, H.H., Egamberdieva, D., Kamilova, F., Lugtenberg, B., Kuo, C.H., 2015. Genome analysis of *Pseudomonas fluorescens* PCL1751: a rhizobacterium that

- controls root diseases and alleviates salt stress for its plant host. *PLoS One* 10 (10), e0140231.
- Choudhury, F.K., Rivero, R.M., Blumwald, E., Mittler, R., 2017. Reactive oxygen species, abiotic stress and stress combination. *Plant J* 90 (5), 856–867.
- Coleman-Derr, D., Tringe, S.G., 2014. Building the crops of tomorrow: advantages of symbiont-based approaches to improving abiotic stress tolerance. *Front Microbiol* 5, 283.
- Damodaran, T., Rai, R.B., Jha, S.K., Kannan, R., Pandey, B.K., Sah, V., et al., 2014. Rhizosphere and endophytic bacteria for induction of salt tolerance in gladiolus grown in sodic soils. *J. plant Interact.* 9 (1), 577–584.
- Dimkpa, C., Weinand, T., Asch, F., 2009. Plant–rhizobacteria interactions alleviate abiotic stress conditions. *Plant Cell Environ* 32 (12), 1682–1694.
- Egamberdieva, D., Kucharova, Z., Davranov, K., Berg, G., Makarova, N., Azarova, T., et al., 2011. Bacteria able to control foot and root rot and to promote growth of cucumber in salinated soils. *Biol Fert Soils* 47 (2), 197–205.
- Erice, G., Ruiz-Lozano, J.M., Zamarreño, Á.M., García-Mina, J.M., Aroca, R., 2017. Transcriptomic analysis reveals the importance of JA-Ile turnover in the response of *Arabidopsis* plants to plant growth promoting rhizobacteria and salinity. *Environ Exp Bot* 143, 10–19.
- Fasciglione, G., Casanovas, E.M., Yommi, A., Sueldo, R.J., Barassi, C.A., 2012. *Azospirillum* improves lettuce growth and transplant under saline conditions. *J Sci Food Agric* 92 (12), 2518–2523.
- Fluhr, R., Mattoo, A.K., Dilley, D.R., 1996. Ethylene—biosynthesis and perception. *Crit Rev Plant Sci* 15 (5–6), 479–523.
- Forni, C., Duca, D., Glick, B.R., 2017. Mechanisms of plant response to salt and drought stress and their alteration by rhizobacteria. *Plant Soil* 410 (1–2), 335–356.
- Foyer, C.H., Noctor, G., 2005. Oxidant and antioxidant signalling in plants: a re-evaluation of the concept of oxidative stress in a physiological context. *Plant Cell Environ* 28 (8), 1056–1071.
- Gamalero, E., Berta, G., Massa, N., Glick, B.R., Lingua, G., 2010. Interactions between *Pseudomonas putida* UW4 and *Gigaspora rosea* BEG9 and their consequences for the growth of cucumber under salt-stress conditions. *J. Appl. Microbiol.* 108 (1), 236–245.
- Gamalero, E., Glick, B.R., 2015. Bacterial modulation of plant ethylene levels. *Plant Physiol* 169 (1), 13–22.
- Gill, S.S., Tuteja, N., 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant. Physiol. Biochem.* 48 (12), 909–930.
- Giovannoni, J., 2001. Molecular biology of fruit maturation and ripening. *Annu Rev Plant Biol* 52 (1), 725–749.
- Glick, B.R., 2005. Modulation of plant ethylene levels by the bacterial enzyme ACC deaminase. *FEMS Microbiol Lett* 251 (1), 1–7.
- Glick, B.R., Cheng, Z., Czarny, J., Duan, J., 2007. Promotion of plant growth by ACC deaminase-containing soil bacteria. *Eur. J. Plant. Pathol.* 119, 329–339.
- Glick, B.R., 2014. Bacteria with ACC deaminase can promote plant growth and help to feed the world. *Microbiol Res* 169 (1), 30–39.
- Grover, M., Ali, S.Z., Sandhya, V., Rasul, A., Venkateswarlu, B., 2011. Role of microorganisms in adaptation of agriculture crops to abiotic stresses. *World J Microbiol Biotechnol* 27 (5), 1231–1240.
- Gururani, M.A., Upadhyaya, C.P., Baskar, V., Venkatesh, J., Nookaraju, A., Park, S.W., 2013. Plant growth-promoting rhizobacteria enhance abiotic stress tolerance in *Solanum tuberosum* through inducing changes in the expression of ROS-scavenging enzymes and improved photosynthetic performance. *J Plant Growth Regul* 32 (2), 245–258.

- Habib, S.H., Kausar, H., Saud, H.M., 2016. Plant growth-promoting rhizobacteria enhance salinity stress tolerance in okra through ROS-scavenging enzymes. *Biomed. Res. Int.* 2016, Article ID 6284547, 10 pages.
- Han, H.S., Lee, K.D., 2005. Plant growth promoting rhizobacteria effect on antioxidant status, photosynthesis, mineral uptake and growth of lettuce under soil salinity. *Res. J. Agric. Biol. Sci.* 1 (3), 210–215.
- Han, Y., Wang, R., Yang, Z., Zhan, Y., Ma, Y., Ping, S., et al., 2015. 1-Aminocyclopropane-1-carboxylate deaminase from *Pseudomonas stutzeri* A1501 facilitates the growth of rice in the presence of salt or heavy metals. *J Microbiol Biotechnol* 25 (7), 1119–1128.
- Heidari, M., Golpayegani, A., 2012. Effects of water stress and inoculation with plant growth promoting rhizobacteria (PGPR) on antioxidant status and photosynthetic pigments in basil (*Ocimum basilicum* L.). *J Saudi Soc Agric Sci* 11 (1), 57–61.
- Ilangumaran, G., Smith, D.L., 2017. Plant growth promoting rhizobacteria in amelioration of salinity stress: a systems biology perspective. *Front Plant Sci* 8, 1768.
- Iqbal, N., Trivellini, A., Masood, A., Ferrante, A., Khan, N.A., 2013. Current understanding on ethylene signaling in plants: the influence of nutrient availability. *Plant Physiol Biochem* 73, 128–138.
- Jha, Y., Subramanian, R.B., Patel, S., 2011. Combination of endophytic and rhizospheric plant growth promoting rhizobacteria in *Oryza sativa* shows higher accumulation of osmoprotectant against saline stress. *Acta Physiol Plant* 33 (3), 797–802.
- Jung, J.K., McCouch, S., 2013. Getting to the roots of it: genetic and hormonal control of root architecture. *Front Plant Sci* 4, 186.
- Kang, S.M., Radhakrishnan, R., Khan, A.L., Kim, M.J., Park, J.M., Kim, B.R., et al., 2014. Gibberellin secreting rhizobacterium, *Pseudomonas putida* H-2-3 modulates the hormonal and stress physiology of soybean to improve the plant growth under saline and drought conditions. *Plant Physiol Biochem* 84, 115–124.
- Kang, S.M., Radhakrishnan, R., Lee, S.M., Park, Y.G., Kim, A.Y., Seo, C.W., et al., 2015. *Enterobacter* sp. SE992-induced regulation of amino acids, sugars, and hormones in cucumber plants improves salt tolerance. *Acta Physiol Plant* 37 (8), 1–10.
- Kapoor, D., Sharma, R., Handa, N., Kaur, H., Rattan, A., Yadav, P., et al., 2015. Redox homeostasis in plants under abiotic stress: role of electron carriers, energy metabolism mediators and proteinaceous thiols. *Front. Environ. Sci.* 3, 13.
- Kende, H., 1993. Ethylene biosynthesis. *Annu Rev Plant Biol* 44 (1), 283–307.
- Kim, K., Jang, Y.J., Lee, S.M., Oh, B.T., Chae, J.C., Lee, K.J., 2014. Alleviation of salt stress by *Enterobacter* sp. EJ01 in tomato and *Arabidopsis* is accompanied by up-regulation of conserved salinity responsive factors in plants. *Mol Cells* 37 (2), 109.
- Kishore, G.K., Pande, S., Podile, A.R., 2006. *Pseudomonas aeruginosa* GSE 18 inhibits the cell wall degrading enzymes of *Aspergillus niger* and activates defence-related enzymes of groundnut in control of collar rot disease. *Australas Plant Pathol* 35 (2), 259–263.
- Klee, H.J., 1993. Ripening physiology of fruit from transgenic tomato (*Lycopersicon esculentum*) plants with reduced ethylene synthesis. *Plant Physiol* 102 (3), 911–916.
- Kloepper, J.W. and Schroth, M.N., August, 1978. Plant growth-promoting rhizobacteria on radishes. In *Proceedings of the 4th international conference on plant pathogenic bacteria* (Vol. 2, pp. 879–882).
- Kloepper, J.W., Schroth, M.N., 1981. Relationship of in vitro antibiosis of plant growth-promoting rhizobacteria to plant growth and the displacement of root microflora. *Phytopathology* 71 (10), 1020–1024.
- Kohler, J., Hernández, J.A., Caravaca, F., Roldán, A., 2009. Induction of antioxidant enzymes is involved in the greater effectiveness of a PGPR versus AM fungi with respect to increasing the tolerance of lettuce to severe salt stress. *Environ Exp Bot* 65 (2), 245–252.

- Lee, G.W., Lee, K.J., Chae, J.C., 2016. *Herbaspirillum* sp. strain GW103 alleviates salt stress in *Brassica rapa* L. ssp. *pekinensis*. *Protoplasma* 253 (3), 655–661.
- Lei, P., Xu, Z., Liang, J., Luo, X., Zhang, Y., Feng, X., et al., 2016. Poly (γ -glutamic acid) enhanced tolerance to salt stress by promoting proline accumulation in *Brassica napus* L. *Plant Growth Regul.* 78 (2), 233–241.
- Li, H., Lei, P., Pang, X., Li, S., Xu, H., Xu, Z., et al., 2017. Enhanced tolerance to salt stress in canola (*Brassica napus* L.) seedlings inoculated with the halotolerant *Enterobacter cloacae* HSNJ4. *Appl Soil Ecol* 119, 26–34.
- Liang, W., Ma, X., Wan, P., Liu, L., 2018. Plant salt-tolerance mechanism: a review. *Biochem Biophys Res Commun* 495 (1), 286–291.
- Liu, S., Hao, H., Lu, X., Zhao, X., Wang, Y., Zhang, Y., et al., 2017. Transcriptome profiling of genes involved in induced systemic salt tolerance conferred by *Bacillus amyloliquefaciens* FZB42 in *Arabidopsis thaliana*. *Sci Rep* 7 (1), 10795.
- Liu, Y., Shi, Z., Yao, L., Yue, H., Li, H., Li, C., 2013. Effect of IAA produced by *Klebsiella oxytoca* Rs-5 on cotton growth under salt stress. *J Gen Appl Microbiol* 59 (1), 59–65.
- Lugtenberg, B., Kamilova, F., 2009. Plant-growth-promoting rhizobacteria. *Annu. Rev. Microbiol.* 63, 541–556.
- Mayak, S., Tirosh, T., Glick, B.R., 2004a. Plant growth-promoting bacteria that confer resistance to water stress in tomato and pepper. *Plant Sci.* 166, 525–530.
- Mayak, S., Tirosh, T., Glick, B.R., 2004b. Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. *Plant Physiol Biochem* 42 (6), 565–572.
- Miller, G., Suzuki, N., Ciftci-Yilmaz, S., Mittler, R., 2010. Reactive oxygen species homeostasis and signalling during drought and salinity stresses. *Plant Cell Environ* 33, 453–467.
- Morgan, P.W., Drew, M.C., 1997. Ethylene and plant responses to stress. *Physiol. Plant.* 100 (3), 620–630.
- Munns, R., 2002. Comparative physiology of salt and water stress. *Plant, cell Environ.* 25 (2), 239–250.
- Munns, R., Tester, M., 2008. Mechanisms of salinity tolerance. *Annu Rev Plant Biol* 59, 651–681.
- Nachimuthu, V.V., Pandian, B.A., Robin, S., 2017. Role of reactive oxygen species in water-deficit stress response. *Reactive Oxygen Species and Antioxidant Systems in Plants: Role and Regulation under Abiotic Stress*. Springer, Singapore, pp. 283–295.
- Nadeem, S.M., Zahir, Z.A., Naveed, M., Asghar, H.N., Arshad, M., 2010. Rhizobacteria capable of producing ACC-deaminase may mitigate salt stress in wheat. *Soil Sci. Soc. Am. J.* 74 (2), 533–542.
- Nakashima, K., Yamaguchi-Shinozaki, K., 2006. Regulons involved in osmotic stress-responsive and cold stress-responsive gene expression in plants. *Physiol Plant* 126 (1), 62–71.
- Nascimento, F.X., Rossi, M.J., Soares, C.R., McConkey, B.J., Glick, B.R., 2014. New insights into 1-aminocyclopropane-1-carboxylate (ACC) deaminase phylogeny, evolution and ecological significance. *PLoS One* 9 (6), e99168.
- Nautiyal, C.S., Srivastava, S., Chauhan, P.S., Seem, K., Mishra, A., Sopory, S.K., 2013. Plant growth-promoting bacteria *Bacillus amyloliquefaciens* NBRISN13 modulates gene expression profile of leaf and rhizosphere community in rice during salt stress. *Plant. Physiol. Biochem.* 66, 1–9.
- Noctor, G., Gomez, L., Vanacker, H., Foyer, C.H., 2002. Interactions between biosynthesis, compartmentation and transport in the control of glutathione homeostasis and signalling. *J Exp Bot* 53 (372), 1283–1304.
- O'donnell, P.J., Jones, J.B., Antoine, F.R., Ciardi, J., Klee, H.J., 2001. Ethylene-dependent salicylic acid regulates an expanded cell death response to a plant pathogen. *Plant J.* 25 (3), 315–323.

- Pii, Y., Mimmo, T., Tomasi, N., Terzano, R., Cesco, S., Crecchio, C., 2015. Microbial interactions in the rhizosphere: beneficial influences of plant growth-promoting rhizobacteria on nutrient acquisition process. A review. *Biol Fert Soils* 51 (4), 403–415.
- Pinedo, I., Ledger, T., Greve, M., Poupin, M.J., 2015. *Burkholderia phytofirmans* PsJN induces long-term metabolic and transcriptional changes involved in *Arabidopsis thaliana* salt tolerance. *Front Plant Sci* 6, 466.
- Porcel, R., Barea, J.M., Ruiz-Lozano, J.M., 2003. Antioxidant activities in mycorrhizal soybean plants under drought stress and their possible relationship to the process of nodule senescence. *New Phytol* 157 (1), 135–143.
- Qin, S., Zhang, Y.J., Yuan, B., Xu, P.Y., Xing, K., Wang, J., et al., 2014. Isolation of ACC deaminase-producing habitat-adapted symbiotic bacteria associated with halophyte *Limonium sinense* (Girard) Kuntze and evaluating their plant growth-promoting activity under salt stress. *Plant Soil* 374 (1–2), 753–766.
- Radhakrishnan, R., Baek, K.H., 2017. Physiological and biochemical perspectives of non-salt tolerant plants during bacterial interaction against soil salinity. *Plant Physiol Biochem* 116, 116–126.
- Robison, M.M., Griffith, M., Pauls, K.P., Glick, B.R., 2001. Dual role for ethylene in susceptibility of tomato to *Verticillium* wilt. *J Phytopathol* 149 (7–8), 385–388.
- Sadrnia, M., Maksimava, N., Khromsova, E., Stanislavich, S., Owlia, P., Arjomandzadegan, M., 2011. Study of the effect of bacterial 1-aminocyclopropane-1-carboxylate deaminase (ACC) deaminase on resistance to salt stress in tomato plant. *Anal. Univ. Oradea. Fas. Biol.* 18, 120–123.
- Sahoo, R.K., Ansari, M.W., Pradhan, M., Dangar, T.K., Mohanty, S., Tuteja, N., 2014. A novel *Azotobacter vinelandii* (SRI Az 3) functions in salinity stress tolerance in rice. *Plant Signal Behav* 9 (7), 511–523.
- Saleem, M., Arshad, M., Hussain, S., Bhatti, A.S., 2007. Perspective of plant growth promoting rhizobacteria (PGPR) containing ACC deaminase in stress agriculture. *J Ind Microbiol Biotechnol* 34 (10), 635–648.
- Saravanakumar, D., Samiyappan, R., 2007. ACC deaminase from *Pseudomonas fluorescens* mediated saline resistance in groundnut (*Arachis hypogaea*) plants. *J Appl Microbiol* 102 (5), 1283–1292.
- Schaller, G.E., Voesenek, L.A., 2015. Focus on ethylene. *Plant Physiol* 169 (1), 1–2.
- Shao, H.B., Chu, L.Y., Lu, Z.H., Kang, C.M., 2008. Primary antioxidant free radical scavenging and redox signaling pathways in higher plant cells. *Int J Biol Sci* 4 (1), 8.
- Shaharouna, B., Arshad, M., Zahir, Z.A., 2006. Effect of plant growth promoting rhizobacteria containing ACC-deaminase on maize (*Zea mays* L.) growth under axenic conditions and on nodulation in mung bean (*Vigna radiata* L.). *Lett. Appl. Microbiol.* 42 (2), 155–159.
- Sharma, I.P., Chandra, S., Kumar, N., Chandra, D., 2017. PGPR: heart of soil and their role in soil fertility. *Agriculturally Important Microbes for Sustainable Agriculture*. Springer, Singapore, pp. 51–67.
- Siddique, M.A., Glick, B.R., Chauhan, P.S., Jong Yim, W., Sa, T., 2011. Enhancement of growth and salt tolerance of red pepper seedlings (*Capsicum annuum* L.) by regulating stress ethylene synthesis with halotolerant bacteria containing 1-aminocyclopropane-1-carboxylic acid deaminase activity. *Plant Physiol Biochem* 49 (4), 427–434.
- Singh, R.P., Jha, P., Jha, P.N., 2015. The plant-growth-promoting bacterium *Klebsiella* sp. SBP-8 confers induced systemic tolerance in wheat (*Triticum aestivum*) under salt stress. *J Plant Physiol* 184, 57–67.
- Směkalová, V., Doskočilová, A., Komis, G., Šamaj, J., 2014. Crosstalk between secondary messengers, hormones and MAPK modules during abiotic stress signalling in plants. *Biotechnol Adv* 32 (1), 2–11.

- Stearns, J.C., Glick, B.R., 2003. Transgenic plants with altered ethylene biosynthesis or perception. *Biotechnol Adv* 21 (3), 193–210.
- Suarez, C., Cardinale, M., Ratering, S., Steffens, D., Jung, S., Montoya, A.M.Z., et al., 2015. Plant growth-promoting effects of *Hartmanniobacter diazotrophicus* on summer barley (*Hordeum vulgare* L.) under salt stress. *Appl Soil Ecol* 95, 23–30.
- Tank, N., Saraf, M., 2010. Salinity-resistant plant growth promoting rhizobacteria ameliorates sodium chloride stress on tomato plants. *J. Plant Interact.* 5 (1), 51–58.
- Tao, J.J., Chen, H.W., Ma, B., Zhang, W.K., Chen, S.Y., Zhang, J.S., 2015. The role of ethylene in plants under salinity stress. *Front. Plant Sci.* 6, 1059.
- Timmusk, S., Paalme, V., Pavlicek, T., Bergquist, J., Vangala, A., Danilas, T., et al., 2011. Bacterial distribution in the rhizosphere of wild barley under contrasting microclimates. *PLoS. ONE.* 6 (3), e17968.
- Tittabutr, P., Piromyong, P., Longtonglang, A., Noisa-Ngiam, R., Boonkerd, N., Teamroong, N., 2013. Alleviation of the effect of environmental stresses using co-inoculation of mungbean by *Bradyrhizobium* and rhizobacteria containing stress-induced ACC deaminase enzyme. *Soil Sci Plant Nutr* 59 (4), 559–571.
- Tiwari, S., Lata, C., Chauhan, P.S., Nautiyal, C.S., 2016. *Pseudomonas putida* attunes morphophysiological, biochemical and molecular responses in *Cicer arietinum* L. during drought stress and recovery. *Plant. Physiol. Biochem.* 99, 108–117.
- Tripathi, A.K., Mishra, B.M., Tripathi, P., 1998. Salinity stress responses in the plant growth promoting rhizobacteria, *Azospirillum* spp. *J Biosci* 23 (4), 463–471.
- Tripathi, A.K., Nagarajan, T., Verma, S.C., Le Rudulier, D., 2002. Inhibition of biosynthesis and activity of nitrogenase in *Azospirillum brasilense* Sp7 under salinity stress. *Curr Microbiol* 44 (5), 363–367.
- Tsuchisaka, A., Theologis, A., 2004. Unique and overlapping expression patterns among the *Arabidopsis* 1-amino-cyclopropane-1-carboxylate synthase gene family members. *Plant Physiol* 136 (2), 2982–3000.
- Upadhyay, S.K., Singh, J.S., Saxena, A.K., Singh, D.P., 2012. Impact of PGPR inoculation on growth and antioxidant status of wheat under saline conditions. *Plant Biol* 14 (4), 605–611.
- Vardharajula, S., Zulfikar Ali, S., Grover, M., Reddy, G., Bandi, V., 2011. Drought-tolerant plant growth promoting *Bacillus* spp.: effect on growth, osmolytes, and antioxidant status of maize under drought stress. *J. Plant Interact.* 6 (1), 1–14.
- Venkateswarlu, B., Desai, S., Prasad, Y.G., 2008. Agriculturally important microorganisms for stressed ecosystems: challenges in technology development and application. In: Khachatourians, G.G., Arora, D.K., Rajendran, T.P., Srivastava, A.K. (Eds.), *Agriculturally Important Microorganisms*, vol. 1. Academic World, Bhopal, pp. 225–246.
- Wang, L., Qin, L., Liu, W., Zhang, D., Wang, Y., 2014. A novel ethylene-responsive factor from *Tamarix hispida*, ThERF1, is a GCC-box-and DRE-motif binding protein that negatively modulates abiotic stress tolerance in *Arabidopsis*. *Physiol. Plant.* 152 (1), 84–97.
- Wani, S.H., Kumar, V., Shriram, V., Sah, S.K., 2016. Phytohormones and their metabolic engineering for abiotic stress tolerance in crop plants. *Crop J* 4 (3), 162–176.
- Xu, J., Xing, X.J., Tian, Y.S., Peng, R.H., Xue, Y., Zhao, W., et al., 2015. Transgenic *Arabidopsis* plants expressing tomato glutathione S-transferase showed enhanced resistance to salt and drought stress. *PLoS One* 10 (9), e0136960.
- Yan, J., Smith, M.D., Glick, B.R., Liang, Y., 2014. Effects of ACC deaminase containing rhizobacteria on plant growth and expression of Toc GTPases in tomato (*Solanum lycopersicum*) under salt stress. *Botany* 92 (11), 775–781.
- Yang, S.F., Hoffman, N.E., 1984. Ethylene biosynthesis and its regulation in higher plants. *Annu Rev Plant Physiol* 35 (1), 155–189.

- Yasumura, Y., Pierik, R., Kelly, S., Sakuta, M., Voesenek, L.A., Harberd, N.P., 2015. An ancestral role for Constitutive Triple Response 1 (CTR1) proteins in both ethylene and abscisic acid signaling. *Plant Physiol* 169, 283–298.
- Yue, H., Mo, W., Li, C., Zheng, Y., Li, H., 2007. The salt stress relief and growth promotion effect of Rs-5 on cotton. *Plant Soil* 297 (1–2), 139–145.
- Zafar-ul-Hye, M., Farooq, H.M., Zahir, Z.A., Hussain, M., Hussain, A., 2014. Application of ACC-deaminase containing rhizobacteria with fertilizer improves maize production under drought and salinity stress. *Int J Agric Biol* 16, 591–596.

FURTHER READING

- Chen, L., Liu, Y., Wu, G., Veronican Njeri, K., Shen, Q., Zhang, N., et al., 2016. Induced maize salt tolerance by rhizosphere inoculation of *Bacillus amyloliquefaciens* SQR9. *Physiol Plant* 158 (1), 34–44.
- Prasad, R., Kumar, M., Varma, A., 2015. Role of PGPR in soil fertility and plant health. In: *Plant-Growth-Promoting Rhizobacteria (PGPR) and Medicinal Plants*. Springer International Publishing, pp. 247–260.

Role of Plant Growth Promoting Rhizobacteria in Drought Tolerance: Regulating Growth Hormones and Osmolytes

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6.1 INTRODUCTION

All kinds of stresses including biotic and abiotic affect crops in a negative manner. Drought is one of the major limitations to food production worldwide and is expected to cause serious plant growth problems for crops on more than 50% of the earth's arable lands by 2050 (Fita et al., 2015; Vinocur and Altman, 2005). In addition, the world population is expected to reach nine billion by 2050, necessitating continued increases in crop production to assure food security (Ngumbi and Kloepper, 2016). Therefore, finding solutions related to water-related problems such as drought and its impacts on food security are of new interest. In particular, improved plant's tolerance to drought stress and better growth of crops that satisfy food demands under limited water resource availability is a need for now days (Editorial, 2010; Mancosu et al., 2015).

The existence and activity of soil microbes are affected by plant type as well as environmental factors. There are certain group of bacteria which provide beneficial effects on total plant growth, development, and yield, these bacteria are termed as plant growth promoting rhizobacteria (PGPR) (Kloepper and Schroth, 1978). Since these bacteria are recognized as important for improved emergence of seedlings, plant biomass,

and better root system proliferation, a lot of work has been already done on identifying PGPR present in natural ecosystems and development of some bacterial strains for commercial use (Podile and Kishore, 2006). These PGPR must have some distinctive characters: (1) they should be capable enough to colonize the root surface, (2) they should compete, multiply and survive with other microbiota, and (3) they must promote plant growth (Maheshwari et al., 2015; Kloepper, 1996). Therefore, myriad of soil bacterial species associated with rhizosphere which enhance plant growth by a plethora of mechanisms are known as PGPR.

Plant growth promotion mediated by PGPR takes place by the amendments of total microbial community residing in rhizosphere niche through the production of various substances (Kloepper and Schroth, 1981). It is estimated that about 2%–5% of rhizobacteria are plant growth promotive in nature (Ahemad and Kibret, 2014; Kloepper and Schroth, 1981). They promote plant growth either by helping in resource acquisition (nitrogen, phosphorus, and other essential minerals) or moderating plant hormonal levels.

Inoculation of seeds with PGPR could be done by seed bacterization, drench application, or via dual treatment. Bacteria are generally adsorbed onto soil particles by simple ion exchange which enhances better fertility to soil when the soil organisms release inorganic nutrients from the organic reserves for sustaining rapid plant growth. Bacteria belong to the genera *Acetobacter*, *Alcaligenes*, *Arthrobacter*, *Azospirillum*, *Azoarcus*, *Azotobacter*, *Beijerinckia*, *Bacillus*, *Burkholderia*, *Derxia*, *Gluconacetobacter*, *Herbaspirillum*, *Klebsiella*, *Ochrobactrum*, *Pantoea*, *Pseudomonas*, *Serratia*, *Stenotrophomonas*, and *Zoogloea* have been subject of extensive studies for their followed mechanisms via which they improve plant growth and yield (Babalola, 2010; Kumar et al., 2014; 2016).

PGPR compensate for the plant growth reduction caused by various types of stresses (Babalola et al., 2007) including, water logging (Barnawal et al., 2012), drought stress (Zahir et al., 2008), heavy metals (Kumar et al., 2009), salt stress (Kaymak et al., 2009), and some other unfavorable environmental conditions. The inoculation of PGPR alleviates plant stress by encouraging beneficial effects on plant health, growth, and accelerating nutrient availability and assimilation. Thus, in the quest to improve soil fertility and crop yield under stressed environmental conditions, there is a need to exploit PGPR for continued beneficial agricultural purposes.



6.2 DROUGHT ADAPTATIONS BY PLANTS-MICROBE COMBINATION

For enduring drought stress plants possess many adaptive traits and root system architecture most important among them (Huang et al., 2014). The architecture of root system consists of root system topology, spatial distribution of primary as well as lateral roots, length and number of various secondary roots. Roots have morphological plasticity depends upon soil physical conditions (Tuberosa, 2012). PGPR inoculation to the plants has been reported for promoting root growth and also altering the architecture of the roots (Gouda et al., 2017; Ngumbi, 2011; Vacheron et al., 2013). PGPR treated alterations in root architecture may further lead to an increase in total root surface area that consequently lead to enhanced water and nutrient uptake, having positive effects on total plant growth and health (Somers et al., 2004; Timmusk et al., 2014). During a study in maize, Nadeem and Bane (2014) used *Alcaligenes facialis* strain treated seeds in growth chambers. Three weeks after plantation, water stressed *A. facialis* inoculated plants demonstrated an increase in root length by 10% in comparison with drought stressed untreated control plants. They explained that due to the PGPR treatment, development of root system occurred and it further led to an improvement in water uptake that allowed inoculated plants to tolerate drought stress. Naveed et al. (2014) reported that maize plants inoculated with *Burkholderia phytofirmans* had significantly increased root biomass.

During drought conditions, plants treated with effective PGPR strains could be able to maintain near-normal growth rates of shoot that consequently results in increased crop productivity. Vardharajula et al. (2011) reported that corn plants inoculation with plant growth-promoting *Bacillus* spp. can improve shoot growth. Under drought stress, all the corn plants inoculated with the tested *Bacillus* spp. obtained significant better shoot length as well as dry biomass in comparison with noninoculated plants. Timmusk et al. (2014) found that during drought stress, PGPR treated wheat plants had 78% higher biomass compared to nontreated plants, confirming the PGPR potential for improving plant performance. Furthermore, another scientist duo, Lim and Kim (2013) demonstrated that drought stressed pepper plants, when inoculated with *Bacillus licheniformis*, had 50% higher biomass than noninoculated plants. The shoot length of plant was also enhanced. As a result of PGPR treatments, improvements in shoot lengths and overall growth of plants exposed to

drought stress have also been reported in other crops including sunflower (Castillo et al., 2013), *Sorghum bicolor* (Grover et al., 2014), tomato (Calvo-Polanco et al., 2016), wheat (Arzanesh et al., 2011; Kasim et al., 2013), chickpea (Tiwari et al., 2016), *Vigna radiata* (Saravanakumar et al., 2011), *Vigna radiata* (Sarma and Saikia, 2014), and maize (García et al., 2017; Naseem and Bano, 2014; Naveed et al., 2014). Altogether, the above mentioned studies evidently indicate that plant treatment with selected PGPR strains leads to increase in root length and shoot growth that actually help plants to tolerate drought stress. More extensive mechanism based studies are still needed to investigate the actual correlation between PGPR-mediated root architecture improvement and drought stress tolerance. Moreover, structural and functional modeling studies of root systems interacting with their soil environment could also be very relevant (Dunbabin et al., 2013).



6.3 MECHANISMS FOLLOWED BY PLANTS FOR DROUGHT TOLERANCE

Drought stress is a multidimensional stress and it obstructs the plant water relations on cellular as well as whole plant levels. Plant selection for crop domestication is generally carried out according to their water level tolerance (Xoconostle-Cázares et al., 2011). There are a number of parameters that are required for crop performance under drought stress. The water use efficiency (WUE) of the crop is the most important parameter. WUE of plants is dependent on transpirational rate and relative growth rate of the plant. Acclimatization tendency of the plant shows the level of resistance under stress conditions (Valladares et al., 2007). Another parameter is amount of reactive oxygen species (ROS) generated during stress. Under drought condition, plant leaves have enough antioxidant enzymes and metabolites, which can manage a large amount of generated ROS and can lower oxidative damage (Zlatev and Lidon, 2012). There are many low molecular weight antioxidants which are produced in plant tissues like glutathione, tocopherols, ascorbic acids, and carotenoids. They all have specific character for reacting with all sorts of reactive oxygen species. The usual characteristic against stress can be enhanced glutathione concentration (for oxygen free radical scavenging), proline (for osmotic regulation), and heat shock proteins (Gill and Tuteja, 2010). These three increase in combination under different stressor. There are a number of

mechanisms via which varieties of plant protect themselves against drought stress. Some of the mechanisms are described below.

6.3.1 Maintenance of relative water content for plant adaptation

Relative water content (RWC) in plant leaves is one of the best criteria for measurement of plant water status because it plays role in the metabolic activity in plant tissues. Lower RWC reflects turgor loss involved in limited cell expansion and, consequently, reduced plant growth (Castillo et al., 2013). Enhanced RWC could be considered a strategy for drought tolerance enhancement. Screening of PGPR could be done by using RWC as a parameter for their drought stress alleviating potential. Many studies on investigation of PGPR ability, have measured RWC in inoculated and non-inoculated plants for mitigating drought stress (Ngumbi and Kloepper, 2016; Bano et al., 2013; Naveed et al., 2014; Naseem and Bano, 2014). PGPR-containing plants maintained relatively higher RWC compared to noncontaining plants, showing that PGPR strains that improve plants survival under drought stress generally increase RWC in the plants (Ngumbi and Kloepper 2016). The mechanisms behind correlation between enhanced RWC during PGPR treatment under drought stress are yet to be discovered. However, Casanovas et al. (2002) performed a study and demonstrated that high RWC in maize treated with *Azospirillum brasilense* was due to bacterial production of abscisic acid inducing stomatal closure and alleviated drought stress. Improved RWC may be due to alterations in sensitivity of physiological processes like stomatal closure. Such studies emphasize the need to understand mechanisms behind observed bacterial-mediated drought tolerance via enhanced RWC (Dodd et al., 2010).

6.3.2 Generation of less ROS under drought stress

One of the definite consequences of drought stress is higher production of various ROS, such as O_2^- (superoxide radical), H_2O_2 (hydrogen peroxide), and HO (hydroxyl radical) (Helena and Carvalho, 2008; Ngumbi and Kloepper, 2016). These ROS reduce usual plant metabolism via oxidative damage to lipids, proteins, and other types of macromolecules, which ultimately causes cell death (Hasanuzzaman et al., 2014). For avoiding deleterious effects of ROS, plants have enzymatic and nonenzymatic oxidants like scavenging enzymes (Simova-Stoilova et al., 2008). Such enzymes include ascorbate peroxidase (APX), superoxide dismutase (SOD), glutathione reductase (GR), peroxidase (POX), and catalase

(CAT) (Farooq et al., 2009; Gill and Tuteja, 2010; Hasanuzzaman et al., 2014). PGPR can mediate for drought tolerance by involving themselves to scavenging system. PGPR inoculation shows elevation in accumulation of antioxidant enzymes, such as CAT, POX, and POX, serves for minimizing oxidative injury and contributes to the drought tolerance potato plants treated with PGPR strains including *Bacillus pumilus* as well as *Bacillus firmus*, induced an increase in the levels of ROS-scavenging enzymes including APX and CAT (Gururani et al., 2013). The specific activity of CAT was up to 1.8 times higher under drought stress in PGPR-inoculated plants in comparison of noninoculated plants. The enhanced levels of ROS-scavenging enzymes could be the major reason for drought stress tolerance in PGPR-treated potato plants.

6.3.3 Modulation of plant growth regulators

The development and growth of plants is under the control of externally applied plant growth regulators as well as several phytohormones, including auxins, ethylene (ET), abscisic acid (ABA), gibberellins (GAs), and cytokinins (CKs) (Farooq et al., 2009). Auxin, GAs, and CKs promote plant growth while ethylene and abscisic acid inhibit growth (Taiz and Zeiger, 2010). Plants exposed to drought stress, have an increase in the contents of certain substances that inhibit growth and therefore, signaling the plants to regulate their water budget (Farooq et al., 2009). Treatment with PGPR improves plant growth during drought stress by manipulating as well as modifying the phytohormone content (Bresson et al., 2014). Such kind of modifications include decreasing ET production (Glick et al., 1998; Belimov et al., 2009) as well as altering the usual balance of CKs and ABA (Figueiredo et al., 2008; Cohen et al., 2009) or IAA movement and concentration (Contesto et al., 2010). All these changes are related to drought stress tolerance during PGPR application and may further contribute to the studied bacterial-mediated drought tolerance.



6.4 PGPR MEDIATED PHYTOHORMONES IN DROUGHT MITIGATION

Phytohormones are important chemical compounds that are regularly produced in plant systems for their growth and development. Phytohormones follow direct mechanisms by which PGPR promote

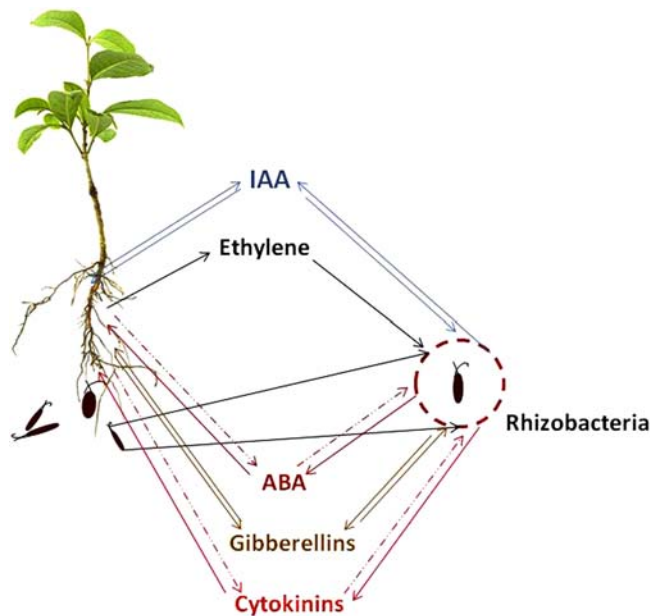


Figure 6.1 Mediation of plant hormone status by rhizobacteria during drought stress. Solid Arrows toward the plant system indicate synthesis of bacterial hormone and solid arrows from the plant system indicate metabolism of bacterial hormone. Dotted lines denote no mechanism of hormonal movement revealed till date (Dodd and Ruiz-Lozano, 2012).

plant growth through production or alteration of plant growth regulators or phytohormones (Fig. 6.1).

Bacterially produced phytohormones contributed to the respiration rate and metabolism of host plant. Inoculation of plants with PGPR can amplify productivity of crops under a drought stress environment (Chanway and Holl, 1994; Barnawal et al., 2017). PGPR possess remarkable potential for modulating the physiological response against water deprivation, thus ensuring plant survival under such stressful conditions (Marasco et al., 2012). PGPR may be effective in imparting osmotic stress tolerance of plants (Boiero et al., 2006). IAA produced by PGPR can modify root system architecture by enhancing the root surface area and number of root tips, thus increases nutrient acquisition and water uptake (Kaushal and Wani, 2016; Mantelin and Touraine, 2004), that further helps plants to survive under water deficit (Egamberdieva and Kucharova, 2009). Physiological modifications in soybean plants inoculated by the gibberellins secreting rhizobacterium *Pseudomonas* improved plant growth under drought conditions (Sang-Mo et al., 2014). Biosynthesis

of ethylene always increases during water deficient conditions and consequently results in reduced root and shoots growth. Extensive studies have been performed for observing certain PGPR strains that possess enzyme ACC (1-aminocyclopropane-1-carboxylate) deaminase (Glick et al., 2007), which can cleave the plant ethylene precursor ACC to ammonia and α -ketobutyrate, thereby lowering the ethylene level (Barnawal et al., 2017; Shaharoon et al., 2006). The deleterious effect of ethylene can be mitigated by the removal of ACC, thus alleviating plant stress and promoting plant growth (Glick et al., 2007; Barnawal et al., 2013; 2017). *Pseudomonas fluorescens* treated rice plants subjected for drought stress have been shown high levels of *COX1*, *PKDP*, *bZIP1*, *AP2-EREBP*, *Hsp20*, and *COC1* gene expression for drought tolerance (Saakre et al., 2017). There are several other examples enlisted that show the important role of PGPR in mitigating drought stress by modulating hormonal levels of various plants (Table 6.1).

6.4.1 IAA producing microbes for drought mitigation

Microorganisms produce auxin phytohormone as indole-3-acetic acid, in the presence of its precursor tryptophan. Auxin generally helps in apical dominance, root growth inhibition, cell enlargement, root initiation, cell division, increased growth rate, phototropism, and geotropism in plants. Eighty percent of microorganisms isolated from the rhizosphere of various crops have the ability to produce auxins as secondary metabolites (Patten and Glick, 2002). Bacteria belonging to the genera *Azospirillum*, *Pseudomonas*, *Xanthomonas*, and *Rhizobium* as well as *A. faecalis*, *Enterobacter cloacae*, *Serratia marcescens*, *Mycobacterium* sp., *Burkholderia*, *Azotobacter*, *Bacillus cereus*, and *Bradyrhizobium japonicum* have been recorded for the production of auxins which help in plant growth stimulation. Diverse metabolic pathways including indole-3- pyruvic acid pathway, indole-3-acetamide pathway, tryptamine pathway, and indole-3- acetonitrile pathway involved in producing bacterial IAA. Some PGPR strains are able to produce $24.6 \mu\text{gml}^{-1}$ of auxins in L-tryptophan presence (Kumar et al., 2015). Plants treated with a PGPR *Pseudomonas putida* can survive under drought stress due to the bacterial production of IAA (Marulanda et al., 2009). Volatile Organic Carbon secreted by *Bacillus subtilis*, causes growth promotion in *Arabidopsis* plants by upregulating transcripts involved in auxin homeostasis (Zhang et al., 2007). *Azospirillum* inoculated wheat seedlings can cope up with severe osmotic stress due to improved morphological modifications in coleoptile xylem architecture (Pereyra et al.,

Table 6.1 List of rhizobacterial strains for modulating phytohormone levels in drought stress in variety of plants

Plant	Rhizobacterial strain	Major effects	Studied by
Pea	<i>Variovorax paradoxus</i> 5C-2	Ethylene, ACC, ABA	Belimov et al. (2009)
Sunflower	<i>Achromobacter xylosoxidans</i> , <i>Bacillus pumilus</i>	Shoot hormone profiles, ABA, SA, JA	Castillo et al. (2013)
Lettuce	<i>Bacillus</i>	Cytokinins	Arkhipova et al. (2007)
Maize	<i>Azospirillum</i>	Gibberellins, ABA	Cohen et al. (2009)
Maize	<i>Variovorax paradoxus</i>	ABA	Dodd et al. (2009)
Maize	<i>Herbaspirillum</i>	Ethylene, ABA	Curá et al. (2017)
Sunflower	<i>Achromobacter xylosoxidans</i> , <i>Alcaligenes</i> , <i>B. pumilus</i>	ABA, jasmonic acid	Forchetti et al. (2007)
Wheat	<i>Azospirillum</i>	ABA	Ilyas and Bano (2010)
Pepper	<i>Bacillus licheniformis</i>	Ethylene	Lim and Kim (2013)
Rice	<i>Bacillus amyloliquefaciens</i>	Ethylene	Kakar et al. (2016)
Trigonella	<i>Bacillus subtilis</i>	Ethylene	Barnawal et al. (2013)
Wheat	<i>Bacillus subtilis</i>	Ethylene, IAA, ABA	Barnawal et al. (2017)
Wheat	<i>Bacillus</i> , <i>Enterobacter</i> , <i>Moraxella</i>	IAA	Raheem et al. (2017)
Potato	<i>Achromobacter xylosoxidans</i> , <i>Pseudomonas oryzae</i> , <i>Variovorax paradoxus</i>	Ethylene, IAA	Belimov et al. (2015)
Alfalfa	<i>Rhizobium</i>	ABA	Defez et al. (2017)
Tomato	<i>Bacillus megaterium</i>	ABA	Porcel et al. (2014)

2012). This was attributed to upregulation of the indole-3-pyruvate decarboxylase gene and enhanced IAA synthesis in *Azospirillum*. *B. subtilis* improves levels of auxin via modulation of auxin responsive genes in wheat under drought stress condition (Barnawal et al., 2017). Chickpea plants inoculated with *P. putida* showed altered response of auxin signaling F-BOX 2(AFB2) (Jatan et al., 2018).

6.4.2 ACC deaminase-containing PGPR in drought tolerance by lowering ethylene levels

Only phytohormone having gaseous nature is ethylene. A synonym commonly used for it, is “wounding hormone” as its production in plant

tissues is easily induced during physical or chemical perturbation. Ethylene production causes root growth inhibition, which is among its myriad of effects on plant growth as well as development. ACC deaminase activity of PGPR would lower ethylene production in host plants roots and result in lengthening of roots (Barnawal et al., 2017). ACC deaminase-containing PGPR strain *Achromobacter piechaudii*, when inoculated in tomato and pepper, significantly increases the fresh and dry weights and confers tolerance against water deficit (Mayak et al., 2004). Treatment of pepper plants with PGPR *B. licheniformis* recorded increased ACC deaminase production, thus imparting tolerance with drought stress (Lim and Kim, 2013). As ethylene is negative regulator of nodulation in legumes, Rhizobia inoculation leads to a temporal stimulation of higher ethylene production that suppresses nodule formation. In contrast, inhibitors of ethylene synthesis or its physiological action promote nodule formation in legumes. The PGPR containing ACC-deaminase can increase nodulation in legumes by degrading and, thus, by lowering ethylene concentration in the plant (Khalid et al., 2017).

A co-relationship between ethylene precursor ACC and IAA demonstrates the positive effects of IAA on root growth by reducing ethylene levels (Lugtenberg and Kamilova, 2009). *Pisum* plants inoculated with ACC deaminase-containing *Pseudomonas* spp. induced better root health leading to an improved water uptake from soil under drought stress (Zahir et al., 2008). An increase in transcripts of genes related to cell division as well as proliferation and also down regulation of genes related to drought stress were observed in canola plants colonized by the ACC deaminase-containing PGPR *E. cloacae* UW4 (Hontzeas et al., 2004). It was further observed that ethylene gene responses were down regulated and those involved auxin responsive genes were upregulated in *Arabidopsis* plants colonized by ACC deaminase-containing *P. fluorescens* (Wang et al., 2005).

6.4.3 PGPR adjusting phytohormone levels other than IAA and ethylene during drought stress

As PGPR play major roles in modulating IAA and ethylene levels in drought tolerance, but their role in altering other hormone levels can never be a matter of negligence. Cytokinins are a class of phytohormones that are involved in cell divisions promotion, cell enlargement, and tissue expansion in various plant parts. Gibberellins are certain class of phytohormones, generally associated with plant morphology modification

through plant tissue extension, particularly stem tissue. Biosynthesis of ABA is induced by cellular dehydration and commonly known as a “stress hormone” because of its enormous accumulation during drought condition. Water loss is generally regulated through ABA by controlling stomatal closure and stress related signal transduction pathways (Yamaguchi-Shinozaki and Shinozaki, 1994). *B. pumilus* and *B. licheniformis* can produce four different forms of GA (Kumar et al., 2015). Production of ABA and gibberellins occurred by *Azospirillum lipoferum* alleviated drought stress in maize plants (Cohen et al., 2009). *Azospirillum* inoculated *Arabidopsis* plants had elevated levels of ABA in comparison with non-inoculated plants (Cohen et al., 2008). A PGPR *Phyllobacterium brassicacearum*, isolated from the rhizospheric soil of *Brassica napus*, enhanced osmotic stress tolerance, when inoculated to *Arabidopsis* plants, by enhancing ABA content, which further leads to lower leaf transpiration (Bresson et al., 2013). Treatment of *Platycladus orientalis* seedlings with *B. subtilis* (having cytokinins producing ability) has been reported for interfering shoot growth suppression, thus conferring water deficit resistance (Liu et al., 2013). Another *Bacillus* sp. has been found in playing different roles in cytokinin signaling in roots and shoots of *Arabidopsis* (Wang et al., 2017).



6.5 OSMOLYTES: BIOMOLECULES TO ENDURE DROUGHT STRESS IN PLANTS

Osmotic adjustment in plant cells is an adaptive mechanism at physiological level which helps to increase the tolerance against drought stress by maintaining cell's turgidity under water deficit conditions (Wani et al., 2013). This osmotic balance is regulated through small molecules termed as osmoprotectants or compatible solutes or osmolytes. These compounds accumulate in cells during osmotic stress without interfering normal metabolic reactions and balance the osmotic difference between the cell's surroundings and the cytosol. Compatible solutes do not interact with proteins and can accumulate at higher concentrations ($>1 \text{ mol kg}^{-1}$ water) without disrupting vital cellular processes and the cellular metabolic machinery. Compatible solutes also function as effective stabilizers of cellular proteins under harsh environmental conditions (Yancey, 2005).

Drought-induced production and accumulation of osmoprotectants in plants has been well studied. Anjum et al. (2017) found that drought stress

triggered the production and accumulation of different osmolytes in three maize hybrid varieties which increased with severity of drought. [Thangella and Rao \(2013\)](#) reported differential accumulation of osmolytes in four cultivars of peanut (*Arachis hypogaea* L.) under drought stress. They observed considerable reduction in RWC in leaves due to drought induced significantly higher content of total leaf protein, free amino acids, proline and total reducing, and soluble sugar. Production of osmolytes as a survival mechanism under harsh environmental conditions is well defined in PGPR. PGPRs' ability of producing various osmoprotectant compounds under desiccation along with plant growth regulators or phytohormones has drawn the attention of researchers worldwide. [Marasco et al. \(2012\)](#) investigated the structure of microbiome associated with drought-sensitive pepper (*Capsicum annuum* L.) for their plant growth promoting potential under water deficit. Microbiome with mixed population of growth promoting and drought tolerant bacteria enhanced photosynthetic activity and biomass (upto 40%) of *C. annuum* under desert farming. Similarly several studies of PGPRs with their plant growth promoting traits and drought tolerant abilities have confirmed their suitability as a bioinoculants under drought stress in various crops ([Table 6.2](#)).



6.6 DIVERSITY OF OSMOPROTECTANTS AMONG PGPR

A wide range of osmoprotective compounds has been identified in plants and microorganisms including sugars, sugar alcohols, amino acids, quaternary ammonium compounds, and tertiary sulphonium compounds. These compounds could be accumulated by microorganisms either through direct acquisition from the environment or through *de novo* biosynthesis ([Galinski, 1995](#)).

6.6.1 Sugar

Sugar molecules such as sucrose, trehalose, fructans etc. have been shown to be accumulated in plants and microorganisms in response to abiotic stresses ([Yuanyuan et al., 2009](#)). Some unusual osmoprotectant sugars including gentiobiose, melibiose, maltose, turanose, raffinose, stachyose, verbascose, altrose, palatinose, and cellobiose are frequently reported in plants ([Panikulangara et al., 2004](#)) which can be catabolized to enhance

Table 6.2 Examples of drought tolerant plant growth promoting bacteria which showed enhanced synthesis of osmolytes during drought stress

Drought-tolerant PGPR strain	Host plant	Osmolytes involved in drought tolerance	Reference
<i>Klebsiella variicola</i> , <i>Raoultella planticola</i> , <i>Pseudomonas fluorescens</i>	Maize	Choline, glycinebetaine	Gou et al. (2015)
<i>Bacillus subtilis</i>	Arabidopsis	Choline, glycinebetaine	Naseem and Bano (2014)
<i>Bacillus amyloliquefaciens</i> , <i>B. licheniformis</i> , <i>B. thuringiensis</i> , <i>Paenibacillus favisporus</i> , <i>B. subtilis</i>	Maize	Free amino acids, proline, total soluble sugars	Vardharajula et al. (2011)
<i>Bacillus subtilis</i>	Timothy	Sugars (sucrose, fructans), amino acids (asparagine, glutamic acid, glutamine), gamma- aminobutyric acid	Gagné-Bourque et al. (2016)
<i>Pseudomonas putida</i>	Maize	Proline	Sandhya et al. (2010)
<i>Bacillus polymyxa</i>	Tomato	Proline	Shintu and Jayaram (2015)
<i>Pseudomonas jessenii</i> , <i>Pseudomonas synxantha</i> , <i>Arthrobacter nitroguajacolicus</i>	Rice	Proline	Gusain et al. (2015)
<i>Rhizobium etli</i>	Bean	Trehalose	Suarez et al. (2008)
<i>Azoapirillum brasilense</i>	Rice	Cadaverine	Cassan et al. (2009)
<i>Burkholderia phytofirmans</i>	Grapevine	Carbohydrates, proline, phenols	Barka et al. (2006)
<i>Bacillus cereus</i> , <i>B. subtilis</i> , <i>Serratia sp.</i>	Cucumber	Proline	Wang et al. (2012)

the accumulation of other osmoprotectants and also functions to stabilize cellular membranes in water deficient conditions (Lokhande and Suprasanna, 2012). Sugar solutes also affect sugar-sensing systems and up or down regulation of variety of genes expression involved in photosynthesis, respiration and in synthesis and degradation of starch and sucrose

(Hare et al., 1998). Trehalose is a chemically unreactive, nonreducing disaccharide which is highly soluble and compatible with cellular metabolism even at high concentrations. Trehalose is an important osmoprotectant in several bacteria and fungi but rare in vascular plants (Lunn et al., 2014, Fernandez et al., 2010).

6.6.2 Sugar alcohols

Sugar alcohols can be categorized into acyclic and cyclic polyols. Their accumulation in cells facilitates osmotic adjustment as well as support redox control. Mannitol, an acyclic polyol, is synthesized from fructose-6-phosphate and reported to have possible role in stabilizing macromolecular structures and promoting scavenging systems for ROS (Llanes et al., 2013). Pinitol, a cyclic polyol is derived from the methylation of myo-inositol (isomer of inositol) and plays an important role as protective and signaling compound (Sureshan et al., 2009). Some of the sugar alcohols (polyols) including glycerol, inositol, mannitol, sorbitol, arabitol, and maltitol protect plant roots and microbial cells from desiccation (Costa et al., 2008; Giri et al., 2013). In case of cellular dehydration, polyol hydroxyl groups effectively replace water in establishing hydrogen bonds and help in maintaining enzyme activities and protection of membrane structures (Noiraud et al., 2001).

6.6.3 Amino acids

It has been well documented that abiotic stresses and desiccation induce accumulation of amino acids (such as proline, alanine, arginine, glycine, amides such as glutamine and asparagine and nonprotein amino acid gamma-aminobutyric acid (GABA), pipecolic acid, citrulline, and ornithine) in higher plants (Mansour, 2000). Proline is the widely reported amino acid with enhanced accumulation in response to drought stress which performs multiple functions within plant cells. It can act as a signaling molecule to modulate mitochondrial functions, influence cell proliferation or cell death, and trigger expression of certain genes, crucial for plant recovery from stress. Exceptional conformational rigidity of proline ensures its high stability under extreme conditions (Szabados and Savoure, 2010).

6.6.4 Quaternary ammonium compounds

Osmoprotectant quaternary ammonium compounds which accumulated in plants include glycine betaine, b-alanine betaine, proline betaine,

choline-O-sulphate, hydroxyproline betaine, and pipercolate betaine (Ashraf and Harris, 2004). Among these, glycine betaine occurs widely in bacteria, cyanobacteria, algae, fungi, and in plants upon prolonged exposure to dehydration due to salinity, drought, heat, and cold stress (Galinski, 1995; Guo et al., 2009; Lokhande and Suprasanna, 2012).

In recent years, transgenic technology and metabolic engineering has been proved very effective in terms of producing new crop varieties which would be more resistant to abiotic stresses. Tolerance to abiotic stresses involves complex metabolic functions which are controlled by many genes. Thus much emphasis has been placed on the biosynthetic pathways of osmoprotectants and associated genes. Research directed toward the application of PGPR in drought affected areas encourages commercialization of inoculants however more advanced information regarding systems biology of plant—microbe interactions in response to environmental stimuli is needed for understanding the regulatory networks of plants and rhizospheric microorganisms.



6.7 CONCLUSION

PGPR can help plants to tolerate drought stress by altering their physiological mechanisms including plant-hormone status. Such PGPR are able to manipulate IAA, ABA, ethylene, cytokines, and GA levels inside plant tissues under stressed situations. Changes in phytohormone levels may lead to better root growth and architecture thus show overall positive effects on plant growth. Productions of osmoprotectants by PGPR are of great importance as they can be used by associated plants for better water uptake under drought conditions. Stress adaptation in plants, induced by rhizobacteria is a combined action of microbial communities in rhizosphere thus microbiome analysis and co-inoculation studies may help in the development of formulations for commercial application to deal with drought stress in agriculture.

REFERENCES

- Ahemad, M., Kibret, M., 2014. Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. *J King Saud Univ Sci* 26 (1), 1–20.
- Valladares, F., Gianoli, E., Gómez, J.M., 2007. Ecological limits to plant phenotypic plasticity. *New Phytol*, 176(4), 749–763.

- Arkhipova, T.N., Prinsen, E., Veselov, S.U., Martinenko, E.V., Melentiev, A.I., Kudoyarova, G.R., 2007. Cytokinin producing bacteria enhance plant growth in dry-ing soil. *Plant Soil* 292 (1–2), 305–315.
- Arzanesh, M.H., Alikhani, H.A., Khavazi, K., Rahimian, H.A., Miransari, M., 2011. Wheat (*Triticum aestivum* L.) growth enhancement by *Azospirillum* sp. under drought stress. *World J Microbiol Biotechnol*, 27(2), 197–205.
- Ashraf, M., Harris, P.J.C., 2004. Potential biochemical indicators of salinity tolerance in plants. *Plant Sci*, 166, 3–16.
- Babalola, O.O., 2010. Beneficial bacteria of agricultural importance. *Biotechnol Lett*, 32, 1559–1570.
- Babalola, O.O., Sanni, A.I., Odhiambo, G.D., Torto, B., 2007. Plant growth-promoting rhizobacteria do not pose any deleterious effect on cowpea and detectable amounts of ethylene are produced. *World J Microbiol Biotechnol*, 23 (6), 747–752.
- Bano, Q.U.D.S.I.A., Ilyas, N., Bano, A., Zafar, N.A.D.I.A., Akram, A.B.I.D.A., Hassan, F., 2013. Effect of *Azospirillum* inoculation on maize (*Zea mays* L.) under drought stress. *Pak. J. Bot.* 45 (S1), 13–20.
- Barka, E.A., Nowak, J., Clement, C., 2006. Enhancement of chilling resistance of inocu-lated grapevine plantlets with a plant growth-promoting rhizobacterium *Burkholderia phytofirmans* strain PsJN. *Appl Environ Microbiol*, 72(11), 7246–7252.
- Barnawal, D., Bharti, N., Maji, D., Chanotiya, C.S., Kalra, A., 2012. 1-Aminocyclopropane-1-carboxylic acid (ACC) deaminase-containing rhizobacteria protect *Ocimum sanctum* plants during waterlogging stress via reduced ethylene genera-tion. *Plant Physiol Biochem* 58, 227–235.
- Barnawal, D., Maji, D., Bharti, N., Chanotiya, C.S., Kalra, A., 2013. ACC deaminase-containing *Bacillus subtilis* reduces stress ethylene-induced damage and improves mycorrhizal colonization and rhizobial nodulation in *Trigonella foenum-graecum* under drought stress. *J Plant Growth Regul* 32 (4), 809–822.
- Barnawal, D., Bharti, N., Pandey, S.S., Pandey, A., Chanotiya, C.S., Kalra, A., 2017. Plant growth promoting rhizobacteria enhances wheat salt and drought stress tolerance by altering endogenous phytohormone levels and TaCTR1/TaDREB2 expression. *Physiol Plant*. Available from: <https://doi.org/10.1111/ppl.12614>.
- Belimov, A.A., Dodd, I.C., Hontzeas, N., Theobald, J.C., Safronova, V.I., Davies, W.J., 2009. Rhizosphere bacteria containing 1-aminocyclopropane-1-carboxylate deami-nase increase yield of plants grown in drying soil via both local and systemic hormone signalling. *New Phytol* 181 (2), 413–423.
- Belimov, A.A., Dodd, I.C., Safronova, V.I., Shaposhnikov, A.I., Azarova, T.S., Makarova, N.M., et al., 2015. Rhizobacteria that produce auxins and contain 1-amino-cyclopro-pane-1-carboxylic acid deaminase decrease amino acid concentrations in the rhizo-sphere and improve growth and yield of well-watered and water-limited potato (*Solanum tuberosum*). *Ann Appl Biol* 167 (1), 11–25.
- Boiero, L., Perrig, D., Masciarelli, O., Penna, C., Cassan, F., Luna, V., 2006. Phytohormone production by three strains of *Bradyrhizobium japonicum*, and possible physiological and technological implications. *Appl Microbiol Biotechnol*, 74, 874–880.
- Bresson, J., Varoquax, F., Bontpart, T., Touraine, B., Vile, D., 2013. The PGPR strain *Phyllobacterium brassicacearum* STM 196 induces a reproductive delay and physiological changes that result in improved drought tolerance in *Arabidopsis*. *New Phytol*, 200, 558–569.
- Bresson, J., Vasseur, F., Dauzat, M., Labadie, M., Varoquax, F., Touraine, B., et al., 2014. Interact to survive: *Phyllobacterium brassicacearum* improves *Arabidopsis* tolerance to severe water deficit and growth recovery. *PLoS One*, 9, e107607.

- Calvo-Polanco, M., Sánchez-Romera, B., Aroca, R., Asins, M.J., Declerck, S., Dodd, I.C., et al., 2016. Exploring the use of recombinant inbred lines in combination with beneficial microbial inoculants (AM fungus and PGPR) to improve drought stress tolerance in tomato. *Environ Exp Bot* 131, 47–57.
- Casanovas, E.M., Barassi, C.A., Sueldo, R.J., 2002. *Azospirillum* inoculation mitigates water stress effects in maize seedlings. *Cereal Res Commun*, 30, 343–350.
- Cassan, F., Maiale, S., Masciarelli, O., Vidal, A., Luna, V., Ruiz, O., 2009. Cadaverine production by *Azospirillum brasilense* and its possible role in plant growth promotion and osmotic stress mitigation. *Eur J Soil Biol*, 45, 12–19.
- Castillo, P., Escalante, M., Gallardo, M., Alemanno, S., Abdala, G., 2013. Effects of bacterial single inoculation and co-inoculation on growth and phytohormone production of sunflower seedlings under water stress. *Acta Physiol Plant*, 35 (7), 2299–2309.
- Chanway, C.P., Holl, F.B., 1994. Growth of outplanted lodgepole pine seedlings one year after inoculation with plant growth promoting rhizobacteria. *For. Sci.* 40 (2), 238–246.
- Cohen, A., Bottini, R., Piccoli, P., 2008. *Azospirillum brasilense* Sp 245 produces ABA in chemically-defined culture medium and increases ABA content in arabidopsis plants. *Plant Growth Regul*, 54, 97–103.
- Cohen, A.C., Travaglia, C.N., Bottini, R., Piccoli, P.N., 2009. Participation of abscisic acid and gibberellins produced by endophytic *Azospirillum* in the alleviation of drought effects in maize. *Botany*, 87, 455–462.
- Contesto, C., Milesi, S., Mantelin, S., Zancarini, A., Desbrosses, G., 2010. The auxin-signaling pathway is required for the lateral root response of *Arabidopsis* to the rhizobacterium *Phyllobacterium brassicacearum*. *Planta*, 232, 1455–1470.
- Costa, P., Bahrman, N., Frigerio, J.M., Kremer, A., Plomion, C., 2008. Water-deficit-responsive proteins in maritime pine. *Plant Mol. Biol.* 38 (4), 587–596.
- Curá, J.A., Franz, D.R., Filosofia, J.E., Balestrasse, K.B., Burgueño, L.E., 2017. Inoculation with *Azospirillum* sp. and *Herbaspirillum* sp. Bacteria Increases the tolerance of maize to drought stress. *Microorganisms* 5 (3), 41.
- Defez, R., Andreozzi, A., Dickinson, M., Charlton, A., Tadini, L., Pesaresi, P., et al., 2017. Improved Drought Stress Response in Alfalfa Plants Nodulated by an IAA Over-producing Rhizobium Strain. *Front Microbiol* 8,2466.
- Dodd, I.C., Jiang, F., Teijeiro, R.G., Belimov, A.A., Hartung, W., 2009. The rhizosphere bacterium *Variovorax paradoxus* 5C-2 containing ACC deaminase does not increase systemic ABA signaling in maize (*Zea mays* L.). *Plant Signal Behav* 4 (6), 519–521.
- Dodd, I.C., Ruiz-Lozano, J.M., 2012. Microbial enhancement of crop resource use efficiency. *Curr. Opin. Biotechnol.* 23 (2), 236–242.
- Dodd, I.C., Zinovkina, N.Y., Safronova, V.I., Belimov, A.A., 2010. Rhizobacterial mediation of plant hormone status. *Ann Appl Biol*, 157(3), 361–379.
- Dunbabin, V.M., Postma, J.A., Schnepf, A., Pagès, L., Javaux, M., Wu, L., et al., 2013. Modelling root–soil interactions using three-dimensional models of root growth, architecture and function. *Plant Soil*, 372, 93–124.
- Editorial, 2010. How to feed a hungry world. *Nature*, 466, 531–532.
- Egamberdieva, D., Kucharova, Z., 2009. Selection for root colonising bacteria stimulating wheat growth in saline soils. *Biol Fert Soils*, 45(6), 561–573.
- Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., Basra, S.M.A., 2009. Plant drought stress: effects, mechanisms and management. *Agron Sustain Dev*, 29(1), 185–212.
- Fernandez, O., Béthencourt, L., Quero, A., Sangwan, R.S., Clement, C., 2010. Trehalose and plant stress responses: friend or foe? *Trends Plant Sci*, 15, 409–417.
- Figueiredo, M.V.B., Buritty, H.A., Martínez, C.R., Chanway, C.P., 2008. Alleviation of drought stress in the common bean (*Phaseolus vulgaris* L.) by co-inoculation with *Paenibacillus polymyxa* and *Rhizobium tropici*. *Appl Soil Ecol*, 40, 182–188.

- Fita, A., Rodríguez-Burruezo, A., Boscaiu, M., Prohens, J., Vicente, O., 2015. Breeding and domesticating crops adapted to drought and salinity: a new paradigm for increasing food production. *Front Plant Sci*, 6, 978.
- Forchetti, G., Masciarelli, O., Alemanno, S., Alvarez, D., Abdala, G., 2007. Endophytic bacteria in sunflower (*Helianthus annuus* L.): isolation, characterization, and production of jasmonates and abscisic acid in culture medium. *Appl Microbiol Biotechnol* 76 (5), 1145–1152.
- Gagné-Bourque, F., Bertrand, A., Claessens, A., Aliferis, K.A., Jabaji, S., 2016. Alleviation of drought stress and metabolic changes in Timothy (*Phleum pratense* L.) colonized with *Bacillus subtilis* B26. *Front Plant Sci*, 7, 584.
- Galinski, E.A., 1995. Osmoadaptation in bacteria. *Adv Microb Physiol*, 37, 273–328.
- García, J.E., Maroniche, G., Creus, C., Suárez-Rodríguez, R., Ramirez-Trujillo, J.A., Groppa, M.D., 2017. In vitro PGPR properties and osmotic tolerance of different *Azospirillum* native strains and their effects on growth of maize under drought stress. *Microbiol Res* 202, 21–29.
- Gill, S.S., Tuteja, N., 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol Biochem*, 48(12), 909–930.
- Giri, J., Dansana, P.K., Kothari, K.S., Sharma, G., Vij, S., Tyagi, A.K., 2013. SAPs as novel regulators of abiotic stress response in plants. *BioEssays* 35 (7), 639–648.
- Glick, B.R., Penrose, D.M., Li, J., 1998. A model for the lowering of plant ethylene concentrations by plant growth-promoting rhizobacteria. *J Theor Biol*, 190(1), 63–68.
- Glick, B.R., Todorovic, B., Czarny, J., Cheng, Z., Duan, J., McConkey, B., 2007. Promotion of plant growth by bacterial ACC-deaminase. *Crit Rev Plant Sci*, 26, 227–242.
- Gou, W., Tian, L., Ruan, Z., Zheng, P., Chen, F., Zhang, L., et al., 2015. Accumulation of choline and glycinebetaine and drought stress tolerance induced in maize (*zea mays*) by three plant growth promoting rhizobacteria (pgpr) strains. *Pak J Bot*, 47 (2), 581–586.
- Gouda, S., Kerry, R.G., Das, G., Paramithiotis, S., Shin, H.S., Patra, J.K., 2017. Revitalization of plant growth promoting rhizobacteria for sustainable development in agriculture. *Microbiol Res* 206, 131–140.
- Grover, M., Madhubala, R., Ali, S.Z., Yadav, S.K., Venkateswarlu, B., 2014. Influence of *Bacillus* spp. strains on seedling growth and physiological parameters of sorghum under moisture stress conditions. *J Basic Microbiol*, 54, 951–961.
- Guo, P., Baum, M., Grando, S., Ceccarelli, S., Bai, G., Li, R., et al., 2009. Differentially expressed genes between drought tolerant and drought-sensitive barley genotypes in response to drought stress during the reproductive stage. *J Exp Bot*, 60(12), 3531–3544.
- Gururani, M.A., Upadhyaya, C.P., Baskar, V., Venkatesh, J., Nookaraju, A., Park, S.W., 2013. Plant growth-promoting rhizobacteria enhance abiotic stress tolerance in *Solanum tuberosum* through inducing changes in the expression of ROS- Scavenging enzymes and improved photosynthetic performance. *J Plant Growth Regul*, 32, 245–258.
- Gusain, Y.S., Singh, U.S., Sharma, A.K., 2015. Bacterial mediated amelioration of drought stress in drought tolerant and susceptible cultivars of rice (*Oryza sativa* L.). *Afr J Biotechnol*, 14(9), 764–773.
- Hare, P.D., Cress, W.A., Van Staden, J., 1998. Dissecting the roles of osmolyte accumulation during stress. *Plant Cell Environ*, 21(6), 535–553.
- Hasanuzzaman, M., Nahar, K., Gill, S.S., Gill, R., Fujita, M., 2014. Drought stress responses in plants, oxidative stress, and antioxidant defense. In: Tuteja, N., Gill, S.S. (Eds.), *Climate Change and Plant Abiotic Stress Tolerance*. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, pp. 209–249.

- Helena, M., Carvalho, C., 2008. Drought stress and reactive oxygen species production, scavenging and signaling. *Plant Signal Behav*, 3, 156–165.
- Hontzeas, N., Saleh, S.S., Glick, B.R., 2004. Changes in gene expression in canola roots induced by ACC-deaminase-containing plant growth promoting bacteria. *Mol Plant Microbe Interact*, 17(8), 865–871.
- Huang, B., DaCosta, M., Jiang, Y., 2014. Research advances in mechanisms of turfgrass tolerance to abiotic stresses: from physiology to molecular biology. *Crit Rev Plant Sci*, 33, 141–189.
- Ilyas, N., Bano, A., 2010. Azospirillum strains isolated from roots and rhizosphere soil of wheat (*Triticum aestivum* L.) grown under different soil moisture conditions. *Biol Fert Soils* 46 (4), 393–406.
- Jatan, R., Chauhan, P.S., Lata, C., 2018. *Pseudomonas putida* modulates the expression of miRNAs and their target genes in response to drought and salt stresses in chickpea (*Cicer arietinum* L.). *Genomics*. Available from: <https://doi.org/10.1016/j.ygeno.2018.01.007>.
- Kakar, K.U., Ren, X.L., Nawaz, Z., Cui, Z.Q., Li, B., Xie, G.L., et al., 2016. A consortium of rhizobacterial strains and biochemical growth elicitors improve cold and drought stress tolerance in rice (*Oryza sativa* L.). *Plant Biol*. 18 (3), 471–483.
- Kasim, W., Osman, M., Omar, M., Abd El-Daim, I., Bejai, S., Meijer, J., 2013. Control of drought stress in wheat using plant-growth promoting rhizobacteria. *J Plant Growth Regul*, 32, 122–130.
- Kaushal, M., Wani, S.P., 2016. Plant-growth-promoting rhizobacteria: drought stress alleviators to ameliorate crop production in drylands. *Ann Microbiol* 66 (1), 35–42.
- Kaymak, H.C., Guvenc, I., Yarali, F., Donmez, M.F., 2009. The effects of bio-priming with PGPR on germination of radish (*Raphanus sativus* L.) seeds under saline conditions. *Turk J Agri For* 33 (2), 173–179.
- Khalid, A., Ahmad, Z., Mahmood, S., Mahmood, T., Imran, M., 2017. Role of ethylene and bacterial ACC-deaminase in nodulation of legumes. In: Zaidi, A., Khan, M., Musarrat, J. (Eds.), *Microbes for Legume Improvement*. Springer, Cham, pp. 95–118.
- Kloepper, J.W., 1996. Host specificity in microbe-microbe interactions. *BioScience* 46 (6), 406–409.
- Kloepper, J.W., Schroth, M.N., 1978. Plant growth promoting rhizobacteria on radishes. In: *Station de pathologie vegetale et phyto-bacteriologie* (Ed.), *Proceedings of the 4th International Conference on Plant Pathogenic Bacteria*, vol. II. Gilbert-Clarey, Tours, France, pp. 879–882.
- Kloepper, J.W., Schroth, M.N., 1981. Relationship of in vitro antibiosis of plant growth-promoting rhizobacteria to plant growth and the displacement of root microflora. *Phytopathology* 71 (10), 1020–1024.
- Kumar, A., Singh, R., Giri, D.D., Singh, P.K., Pandey, K.D., 2014. Effect of *Azotobacter chroococcum* CL13 inoculation on growth and curcumin content of turmeric (*Curcuma longa* L.). *Int J Curr Microbiol Appl Sci* 3 (9), 275–283.
- Kumar, A., Bahadur, I., Maurya, B.R., Raghuwanshi, R., Meena, V.S., Singh, D.K., et al., 2015. Does a plant growth-promoting rhizobacteria enhance agricultural sustainability. *J Pure Appl Microbiol* 9 (1), 715–724.
- Kumar, A., Singh, V., Singh, M., Singh, P.P., Singh, S.K., Singh, P.K., et al., 2016. Isolation of plant growth promoting rhizobacteria and their impact on growth and curcumin content in *Curcuma longa* L. *Biocatalysis and Agriculture*. *Biotechnology* 8, 1–7.
- Kumar, K.V., Srivastava, S., Singh, N., Behl, H.M., 2009. Role of metal resistant plant growth promoting bacteria in ameliorating fly ash to the growth of *Brassica juncea*. *J Hazard Mater*, 170 (1), 51–57.

- Lim, J.H., Kim, S.D., 2013. Induction of drought stress resistance by multi-functional PGPR *Bacillus licheniformis* K11 in pepper. *Plant Pathol J*, 29 (2), 201–208.
- Liu, J., Xia, Z., Wang, M., Zhang, X., Yang, T., Wu, J., 2013. Overexpression of a maize E3 ubiquitin ligase gene enhances drought tolerance through regulating stomatal aperture and antioxidant system in transgenic tobacco. *Plant Physiol Biochem*, 73, 114–120.
- Llanes, A., Bertazza, G., Palacio, G., Luna, V., 2013. Different sodium salts cause different solute accumulation in the halophyte *Prosopis strombulifera*. *Plant Biol (Stuttg)* 1, 118–125.
- Lokhande, V.H., Suprasanna, P., 2012. Prospects of halophytes in understanding and managing abiotic stress tolerance. In: Ahmad, P., Prasad, M.N.V. (Eds.), *Environmental Adaptations and Stress Tolerance of Plants in the Era of Climate Change*. Springer, New York, pp. 29–56.
- Lugtenberg, B., Kamilova, F., 2009. Plant-growth-promoting rhizobacteria. *Ann Rev Microbiol*, 63, 541–556.
- Lunn, J.E., Delorge, I., Figueroa, C.M., Van Dijck, P., Stitt, M., 2014. Trehalose metabolism in plants. *Plant J*, 79, 544–567.
- Maheshwari, D.K., Dheeman, S., Agarwal, M., 2015. Phytohormone-producing PGPR for sustainable agriculture. *Bacterial Metabolites in Sustainable Agroecosystem*. Springer, Cham, pp. 159–182.
- Mancosu, N., Snyder, R.L., Kyriakakis, G., Spano, D., 2015. Water scarcity and future challenges for food production. *Water*, 7, 975–992.
- Mansour, M.M.F., 2000. Nitrogen containing compounds and adaptation of plants to salinity stress. *Biol Plant*, 43, 491–500.
- Mantelin, S., Touraine, B., 2004. Plant growth-promoting rhizobacteria and nitrate availability: impacts on root development and nitrate uptake. *J Exp Bot*, 55, 27–34.
- Marasco, R., Rolli, E., Ettoumi, B., Vigani, G., Mapelli, F., Borin, S., et al., 2012. A drought resistance promoting microbiome is selected by root system under desert farming. *PLoS One* 7, e48479.
- Marulanda, A., Barea, J.M., Azcón, R., 2009. Stimulation of plant growth and drought tolerance by native microorganisms (AM fungi and bacteria) from dry environments: mechanisms related to bacterial effectiveness. *J Plant Growth Regul*, 28, 115–124.
- Mayak, S., Tirosh, T., Glick, B.R., 2004. Plant growth promoting bacteria that confer resistance to water stress in tomato and pepper. *Plant Sci*, 166, 525–530.
- Naseem, H., Bano, A., 2014. Role of plant growth-promoting rhizobacteria and their exopolysaccharide in drought tolerance in maize. *J Plant Interact*, 9, 689–701.
- Naveed, M., Mitter, B., Reichenauer, T.G., Wiczorek, K., Sessitsch, A., 2014. Increased drought stress resilience of maize through endophytic colonization by *Burkholderia phytofirmans* PsJN and *Enterobacter* sp. FD 17. *Environ Exp Bot*, 97, 30–39.
- Ngumbi, E., Kloepper, J., 2016. Bacterial-mediated drought tolerance: current and future prospects. *Appl Soil Ecol*, 105, 109–125.
- Ngumbi, E.N., 2011. Mechanisms of olfaction in parasitic wasps: analytical and behavioral studies of response of a specialist (*Microplitis croceipes*) and a generalist (*Cotesia marginiventris*) parasitoid to host-related odor. Auburn University, Auburn, Ph.D. Dissertation.
- Noiraud, N., Maurousset, L., Lemoine, R., 2001. Transport of polyols in higher plants. *Plant Physiol Biochem*, 39, 717–728.
- Panikulangara, T.J., Eggers-Schumacher, G., Munderlich, M., Stransky, H., Schöffl, F., 2004. Galactinol synthase1. A novel heat shock factor target gene responsible for heat-induced synthesis of raffinose family oligosaccharides in *Arabidopsis*. *Plant Physiol*. 136 (2), 3148–3158.

- Patten, C.L., Glick, B.R., 2002. Role of *Pseudomonas putida* indoleacetic acid in development of the host plant root system. *Appl Environ Microbiol* 68 (8), 3795–3801.
- Pereyra, M.A., Garcia, P., Colabelli, M.N., Barassi, C.A., Creus, C.M., 2012. A better water status in wheat seedlings induced by *Azospirillum* under osmotic stress is related to morphological changes in xylem vessels of the coleoptile. *Appl Soil Ecol*, 53, 94–97.
- Podile, A.R., Kishore, K.G., 2006. Plant growth promoting rhizobacteria (PGPR) to apples increases yield, growth and nutrient element content to leaves. *J Sustain Agric* 30, 145–155.
- Porcel, R., Zamarreño, Á.M., García-Mina, J.M., Aroca, R., 2014. Involvement of plant endogenous ABA in *Bacillus megaterium* PGPR activity in tomato plants. *BMC Plant Biol* 14 (1), 36.
- Raheem, A., Shaposhnikov, A., Belimov, A.A., Dodd, I.C., Ali, B., 2017. Auxin production by rhizobacteria was associated with improved yield of wheat (*Triticum aestivum* L.) under drought stress. *Arch Agron Soil Sci* 64 (4), 574–587.
- Saakre, M., Baburao, T.M., Salim, A.P., Ffancies, R.M., Achuthan, V.P., Thomas, G., et al., 2017. Identification and Characterization of Genes Responsible for Drought Tolerance in Rice Mediated by *Pseudomonas fluorescens*. *Ric Sci* 24 (5), 291–298.
- Sandhya, V., Ali, S.Z., Grover, M., Reddy, G. and, Venkateswarlu, B., 2010. Effect of plant growth promoting *Pseudomonas* spp. on compatible solutes, antioxidant status and plant growth of maize under drought stress. *Plant Growth Regul*, 62 (1), 21–30.
- Sang-Mo, K., Radhakrishnan, R., Khan, A.L., Min-Ji, K., Jae-Man, P., Bo-Ra, K., et al., 2014. Gibberellin secreting rhizobacterium, *Pseudomonas putida* H-2-3 modulates the hormonal and stress physiology of soybean to improve the plant growth under saline and drought conditions. *Plant Physiol Biochem*, 84, 115–124.
- Saravanakumar, D., Kavino, M., Raguchander, T., Subbian, P., Samiyappan, R., 2011. Plant growth promoting bacteria enhance water stress resistance in green gram plants. *Acta Physiol Plant*, 33, 203–209.
- Sarma, R., Saikia, R., 2014. Alleviation of drought stress in mung bean by strain *Pseudomonas aeruginosa* GGRJ21. *Plant Soil*, 377, 111–126.
- Shaharoona, B., Arshad, M., Zahir, Z.A., 2006. Effect of plant growth promoting rhizobacteria containing ACC-deaminase on maize (*Zea mays* L.) growth under axenic conditions and on nodulation in mung bean (*Vigna radiata* L.). *Lett Appl Microbiol*, 42, 155–159.
- Shintu, P.V., Jayaram, K.M., 2015. Phosphate solubilising bacteria (*Bacillus polymyxa*) – An effective approach to mitigate drought in tomato (*Lycopersicon esculentum* Mill.). *Trop Plant Res*, 2(1), 17–22.
- Simova-Stoilova, L., Demirevska, K., Petrova, T., Tsenov, N., Feller, U., 2008. Antioxidative protection in wheat varieties under severe recoverable drought at seedling stage. *Plant Soil Environ*, 54, 529–536.
- Somers, E., Vanderleyden, J., Srinivasan, M., 2004. Rhizosphere bacterial signaling: a love parade beneath our feet. *Crit Rev Microbiol*, 304, 205–240.
- Suarez, R., Wong, A., Ramirez, M., Barraza, A., OrozcoMdel, C., Cevallos, M.A., et al., 2008. Improvement of drought tolerance and grain yield in common bean by over expressing trehalose-6-phosphate synthase in rhizobia. *Mol Plant Microbe Interact* 21, 958–966.
- Sureshan, K.M., Murakami, T. and, Watanabe, Y., 2009. Total syntheses of cyclitol based natural products from myo-inositol: brahol and pinpollitol. *Tetrahedron*, 65, 3998–4006.
- Szabados, L., Savoure, A., 2010. Proline: a multifunctional amino acid. *Trends Plant Sci*, 15, 89–97.
- Taiz, L., Zeiger, E., 2010. *Plant Physiology*, fifth ed. Sinauer Associates Inc, Massachusetts.

- Thangella, A.V.P., Rao, D.M., 2013. Differential Accumulation of Osmolytes in 4 Cultivars of Peanut (*Arachis hypogaea* L.) under Drought Stress. *J Crop Sci Biotechnol* 16 (2), 151–159.
- Timmusk, S., Abd El-Daim, I.A., Copolovici, L., Tanilas, T., Kannaste, A., Behers, L., et al., 2014. Drought-tolerance of wheat improved by rhizosphere bacteria from harsh environments: enhanced biomass production and reduced emissions of stress volatiles. *PLoS One*, 9, e96086.
- Tiwari, S., Lata, C., Chauhan, P.S., Nautiyal, C.S., 2016. *Pseudomonas putida* attunes morphophysiological, biochemical and molecular responses in *Cicer arietinum* L. during drought stress and recovery. *Plant Physiol Biochem* 99, 108–117.
- Tuberosa, R., 2012. Phenotyping for drought tolerance of crops in the genomic era. *Front Physiol*, 3, 10–20.
- Vacheron, J., Desbrosses, G., Bouffaud, M., Touraine, B., Moenne-Loccoz, Y., Muller, D., et al., 2013. Plant growth- promoting rhizobacteria and root system functioning. *Front Plant Sci*, 4, 356.
- Vardharajula, S., Ali, S.Z., Grover, M., Reddy, G., Bandi, V., 2011. Drought-tolerant plant growth promoting *Bacillus* spp.: effect on growth osmolytes, and antioxidant status of maize under drought stress. *J Plant Interact*, 6, 1–14.
- Vinocur, B., Altman, A., 2005. Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations. *Curr Opin Biotechnol* 16, 123–132.
- Wang, C.J., Yang, W., Wang, C., Gu, C., Niu, D.D., Liu, H.X., et al., 2012. Induction of drought tolerance in cucumber plants by a consortium of three plant growth-promoting rhizobacterium strains. *PLoS One*, 7, 1–10.
- Wang, J., Zhang, Y., Jin, J., Li, Q., Zhao, C., Nan, W., et al., 2017. An intact cytokinin-signaling pathway is required for *Bacillus* sp. LZR216-promoted plant growth and root system architecture alteration in *Arabidopsis thaliana* seedlings. *Plant Growth Regul* 84 (3), 507–518.
- Wang, Y., Ohara, Y., Nakayashiki, H., Tosa, Y., Mayama, S., 2005. Microarray analysis of the gene expression profile induced by the endophytic plant growth promoting rhizobacteria, *Pseudomonas fluorescens* FPT9601-T5 in *Arabidopsis*. *Mol Plant Microbe Interact* 18, 385–396.
- Wani, H., Shabir, Singh, N.B., Haribhushan, A., Mir, J.I., 2013. Compatible solute engineering in plants for abiotic stress tolerance-role of glycine betaine. *Curr. Genomics* 14 (3), 157–165.
- Xoconostle-Cázares, B., Ramírez-Ortega, F.A., Flores-Elenes, L., Ruiz-Medrano, R., 2011. Drought tolerance in crop plants. *Amer J Plant Physiol* 5, 241–256.
- Yamaguchi-Shinozaki, K., Shinozaki, K., 1994. A novel cis-acting element in an *Arabidopsis* gene is involved in responsiveness to drought, low-temperature, or high-salt stress. *Plant Cell* 6 (2), 251–264.
- Yancey, P.H., 2005. Organic osmolytes as compatible, metabolic and counteracting cytoprotectants in high osmolarity and other stresses. *J Exp Biol*, 208, 2819–2830.
- Yuan, M., Yali, Z., Jiang, L. and Hongbo, S., 2009. Roles of plant soluble sugars and their responses to plant cold stress. *Afr J Biotechnol*, 8, 2004–2010.
- Zahir, Z.A., Munir, A., Asghar, H.N., Shaharoona, B. and, Arshad, M., 2008. Effectiveness of rhizobacteria containing ACC deaminase for growth promotion of peas (*Pisum sativum*) under drought conditions. *J Microbiol Biotechnol* 18 (5), 958–963.
- Zhang, H., Kim, M.S., Krishnamachari, V., Payton, P., Sun, Y., Grimson, M., Paré, P.W., 2007. Rhizobacterial volatile emissions regulate auxin homeostasis and cell expansion in *Arabidopsis*. *Planta* 226 (4), 839.
- Zlatev, Z., Lidon, F.C., 2012. An overview on drought induced changes in plant growth, water relations and photosynthesis. *Emir J Food Agric* 24 (1), 57.



Plant Growth Promoting Rhizobacteria (PGPR) for Sustainable Agriculture: Perspectives and Challenges

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7.1 INTRODUCTION

The world's agricultural system in the twenty-first century faces more challenges, like decline in productivity and degradation in agro-ecosystem sustainability. As per the United Nations estimates, the global human population is projected to reach ~9 billion by 2050 (Wood, 2001) and there has been a continuous increase in the demand for food, and a simultaneous scarcity in supply (Alexandratos and Bruinsma, 2012; Kumar et al., 2017a). According to FAO (2009) report by 2050 to meet projected demand agricultural production will have to rise by 70%. At the same time, people are becoming aware that sustainable agricultural practices are fundamental to meet the future world's agricultural demands (Altieri, 2004).

For sustainable agriculture maintenance soil dynamic nature is of prime importance (Paustian et al., 2016;), not only for sufficient food production but also for maintaining worldwide environmental sustainability for the future generation (Kumar et al., 2017b; Ahmad et al., 2016; Zahedi, 2016). The race for producing more yields of crop by adopting more intensive agronomic practices like application of more inorganic fertilizers and pesticides is thought to have adverse effects on the soil health and environment sustainability (Mahdi et al., 2010; Xiang et al., 2012). Thus the

expansion of agricultural land with fertile soil is near impossible; hence researchers and scientists have sifted their attention for a safer and productive means of agricultural practices. Therefore, exploring nonconventional resources is an urgent need not only to mitigate the demand of ever-increasing population but also to sustain our ecosystem from the further degradation. Sustainability in the agriculture production cannot be attained without microbiological population in soil under the present circumstances (Vaxevanidou et al., 2015; Patil et al., 2014). Among these potential soil microorganisms, bacteria known as plant growth promoting rhizobacteria (PGPR) are the most promising. In this sense, PGPR may be used to enhance plant health and promote plant growth rate without environmental contamination (Calvo et al., 2014; Fernando deAraujo, 2008; Singh et al., 2017a,b).

The role of PGPR with the aim of improving nutrients availability for plants is an important practice and necessary for crop production (Santiago de Freitas et al., 2007; Kumar et al., 2016a,b). PGPR can affect plant growth by different direct and indirect mechanisms (Gupta et al., 2002). They stimulate plant growth through mobilizing nutrients in soils, producing numerous plant growth regulators, protecting plants from phytopathogens by controlling or inhibiting them, improving soil structure and bioremediating the polluted soils by sequestering toxic heavy metal species and degrading xenobiotic compounds like pesticides (Ahemad, 2012; Ahemad and Malik, 2011; Hayat et al., 2010; Rajkumar et al., 2010). The use of PGPR for sustainable and secure agriculture has increased worldwide during the last couple of decades. Several research investigations are conducted on the understanding of the diversity, dynamics and importance of soil PGPR communities and their beneficial and cooperative roles in agricultural productivity (Gray and Smith, 2005; Nogueira da Silva et al., 2006; Figueiredo et al., 2008; Fernando deAraujo, 2008). In this context, there is an ongoing rigorous research worldwide with greater impetus to explore a wide range of rhizobacteria possessing novel traits like heavy metal detoxifying potentials (Ma et al., 2011; Wani and Khan, 2010), pesticide degradation/ tolerance (Ahemad and Khan, 2012), salinity tolerance (Tank and Saraf, 2010; Mayak et al., 2004), biological control of phytopathogens and insects (Hynes et al., 2008; Russo et al., 2008) along with the normal plant growth promoting properties such as, phytohormone (Ahemad and Khan, 2012; Tank and Saraf, 2010), siderophore (Jahanian et al., 2012; Tian et al., 2009), 1-aminocyclopropane- 1-carboxylate, hydrogen cyanate (HCN), and ammonia

production, nitrogenase activity (Glick, 2012; Khan, 2005) phosphate solubilization (Ahemad and Khan, 2012), potassium solubilization etc. Hence, diverse symbiotic (*Rhizobium*, *Bradyrhizobium*, *Mesorhizobium*) and non-symbiotic (*Pseudomonas*, *Bacillus*, *Klebsiella*, *Azotobacter*, *Azospirillum*, *Azomonas*), rhizobacteria are now being used worldwide as bio-inoculants to promote plant growth and development. Thus, based on their activities PGPR can be classified as biofertilizers, phyto-stimulators, rhizoremediators and biopesticides etc. (Somers et al., 2004). This chapter will therefore attempt to shed more light on the role of PGPR to improve nutrient use efficiency (NUE) and crop sustainability simultaneously. The information generated from this could be very beneficial to those who are concerned about environmental protection and agricultural sustainability.



7.2 THE RHIZOSPHERE: A PLAY GROUND FOR PGPR ACTIVITIES

Rhizosphere also known as the microbe storehouse is the soil zone surrounding the plant roots where the biological and chemical features of the soil are influenced by the roots. The rhizosphere is coined more than hundred years ago by Hiltner in 1904. It is a hot spot for microorganisms, where severe, intense interactions take place between the plant, soil, and microfauna (Antoun and Prévost, 2005; Kumar et al., 2015b). They may have positive, negative or neutral effect on plant growth (Ordookhani and Zare, 2011). Plant growth and productivity is highly affected by these interactions. Different type of microorganisms such as bacteria, fungi, protozoa, algae coexist among them. Out of them, plant growth promoting bacteria (PGPR) are most abundant among all others in the rhizosphere. It is well established that the bacterial population in the rhizosphere are higher than in bulk soil (Bahadur et al., 2017; Verma et al., 2017). Plants take up water and nutrients through rhizosphere where microorganisms interact with root exudates. Root exudates include carbohydrates, sugar, organic acids, vitamins, flavonoids, nucleotides, enzymes, hormones, and volatile compounds inorganic ions, gaseous molecules (Table 7.1). The exudates act as messengers that stimulate interactions between roots and soil organisms. Thus, the rhizosphere has emerged as a versatile and dynamic ecological environment in the soil with high microbial diversity.

Table 7.1 Various components under rhizosphere of different crop species

Organic acids	Citric acid, oxalic acid, malic acid, fumaric acid, succinic acid, acetic acid, butyric acid, glycine acid, piscidic acid, formic acid, aconitic acid, lactic acid, pyruvic acid, glutaric acid, malonic acid, aldonic acid, erythronic acid, tetronic acid.
Amino acid	α -alanine, β -alanine, asparagines, cystein, cystine, glutamate, glycine, isoleucine, leucine, lysine, methionine, serine, threonine, proline, valine, tryptophan, ornithine, histidine, arginine, homoserine, phenylalanine, γ -Aminobutyric acid, α -Aminoadipic acid
Sugar	Glucose, fructose, galactose, ribose, xylose, rahamnose, arabinose, desoxyribose, oligosaccharides, raffinose, maltose
Vitamins	Biotin, thiamin, pantothenate, riboflavin, Niacin
Purines/nucleosides	Adenine, guanine, cytidine, uridine Acid/alkaline-
Enzymes	phosphatase, invertase, amylase, Protease
Inorganic ions and gaseous Molecules	HCO_3^- , OH^- , H^+ , CO_2 - H_2

Generally, about 2–5% of rhizosphere bacteria are PGPR (Jha et al., 2012; Sgroey et al., 2009). In the rhizosphere metabolic activities of these bacteria has shown many positive effects on plant growth and developments (Singh et al., 2004). PGPR increased availability and uptakes of nutrients, and disease suppression (Morrissey et al., 2004; Haas and Défago, 2005; Mendes et al., 2011; Kumar et al., 2015b), resistance to abiotic and biotic stresses (Zolla et al., 2013; Badri et al., 2013), all these leads to increases in crop productivity and agricultural sustainability (Bardgett et al., 2014; Nath et al., 2017; Sarkar et al., 2017; Verma et al., 2017).



7.3 WHAT ARE PLANT GROWTH PROMOTING RHIZOBACTERIA

PGPR are a group of bacteria capable to actively colonize the plants root system and improve their growth and yield (Wu et al., 2005). They colonize all ecological niches of root to all stages of plant development, even in the presence of a competing microflora. PGPR represent about

2 to 5% of total rhizospheric bacteria (Antoun and Kloepper., 2001). The term PGPR was proposed by Kloepper et al., 1980 and has been used for a long time, especially for fluorescent *Pseudomonas* involved in the pathogenic biological control and enhancing plant growth. Later, Kapulnik et al., 1981 extended this term to the rhizobacteria capable to directly promote plant growth. Today, the term of PGPR is used to refer to all bacteria living in the rhizosphere and improve plant growth through one or more mechanisms (Haghighi et al., 2011). A wide range of free-living as well as associative and symbiotic rhizobacteria species belonging to the genus *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthrobacter*, *Burkholderia*, *Bacillus* and *Serratia* was reported as PGPR (Saharan and Nehra, 2011). These rhizobacteria can affect the plants growth and development through different ways. Generally, PGPR promote plant growth directly by either increasing nutrient acquisition (nitrogen, phosphorus, potassium and essential minerals) or modulating plant hormone levels, or indirectly by decreasing the inhibitory effects of various pathogens on plant growth and development in the forms of bio-control agents (Glick, 2012). PGPR can also clean environment by detoxifying pollutants like, heavy metals and pesticides. Several studies are still going on to understand the diversity and importance of soil PGPR communities and their roles in betterment of agricultural sustainability. The PGPR effects depend on ecological and soil factors, plant species, plant age, development phase and soil type (Werner, 2000). On the whole, these PGPR bacteria modulate plant-soil chemistry, which in turn pave the way towards the plant growth and agriculture sustainability.



7.4 OCCURRENCE AND FORMS OF PGPR

According to their degree of association with the plant root cells, PGPRs can be classified into extracellular plant growth promoting rhizobacteria (ePGPR) and intracellular plant growth promoting rhizobacteria (iPGPR) (Martínez-Viveros et al., 2010). The ePGPRs inhabit the rhizosphere, on the rhizoplane or in the spaces between the cells of the root cortex whereas iPGPR mainly inhabit inside the specialized nodular structures of root cells. The rhizobacterial genera included as ePGPR *Agrobacterium*, *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*,

Caulobacter, *Chromobacterium*, *Erwinia*, *Flavobacterium*, *Micrococcus*, *Pseudomonas* and *Serratia* (Gray and Smith, 2005). The endophytic microbes belonging to iPGPR include *Allorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, and *Rhizobium*, as well as *Frankia species*, which can fix atmospheric nitrogen specifically for higher plants (Bhattacharyya and Jha, 2012, Verma et al., 2010). Some of the important plant species forming symbiotic association with these rhizobial species includes *Acacia sp.*, *Arachis hypogaea*, *Cajanus cajan*, *Cercis canadensis*, *Cicer arietinum*, *Glycine max*, *Lens culinaris*, *Lotus corniculatus*, *Medicago sativa*, *Phaseolus vulgaris*, *Pisum sativum* and *Trifolium sp.* (Verma et al., 2010). In addition, several actinomycetes, one of the major components of rhizosphere microbial populations are also useful because of their significant ecological roles in soil nutrient cycling (Halder et al., 1991; Elliott and Lynch, 1995) as well in plant growth-promoting activities (Merzaeva and Shirokikh, 2006). Numbers of reports (Gomes et al., 2000; Sousa et al., 2008) are available on the potential of actinomycetes as plant growth-promoting agent. Actinomycetes strains like *Micromonospora sp.*, *Streptomyces sp.*, *Streptosporangium sp.*, and *Thermobifida sp.*, are recorded as best to colonize the plant rhizosphere, showing an immense potentiality as biocontrol agent against a range of root pathogenic fungi (Franco-Correa et al., 2010). Soil actinomycetes are also an important source of diverse antimicrobial metabolites (Terkina et al., 2006).



7.5 ROLE OF PGPR FOR SUSTAINABLE AGRICULTURE

In the recent years, use of biological inoculants for sustainable crop production is attaining popularity in various parts of the world. The PGPR interactions are vital not only for the growth and productivity of the plants but are also imperative to our planet's health and functioning. PGPR enhance plant growth (Table 7.2) due to specific traits (Gupta et al., 2015). PGPR enhance plant growth through direct and indirect mechanisms, which involve enhancing plant physiology and resistance to different phytopathogens through various modes and actions (Zakry et al., 2012). These include nutrient fixation, neutralizing biotic and abiotic stress, and producing volatile organic compounds (VOCs) and enzymes to prevent disease. However, the mode of action of different types of PGPR

Table 7.2 Different modes of action considered for growth promotion by PGPR (plant growth promoting rhizobacteria)

Term	Definition	Mechanism	References
Biofertilizer	A substance which contains live microorganisms which when applied to the seed, plant or the soil, colonizes the rhizosphere or the interior of the plant and promotes the growth through increased supply/or availability of primary nutrients to the host Plant	Phosphate solubilization Siderophores production Exopolysaccharides production Biofixation of atmospheric nitrogen	Vansuyt et al., 2007 Yazdani et al., 2009 Sandhya et al., 2009 Weyens et al., 2010
Phytostimulator	Microorganism with the ability to produce or change the concentration of growth regulators such as IAA, GA, cytokinins and ethylene	Ethylene production Cytokinins production Gibberellins production Indole Acetic Acid production	Glick et al., 2007 Kang et al., 2009 Kang et al., 2009 Ashrafuzzaman et al., 2009
Biopesticides	Microorganisms that promote plant growth through the control of phytopathogenic agents, mainly for the production of antibiotics and antifungal metabolites	Antibiotics production Lytic enzymes production Hydrogen cyanide production Volatile compounds production Induction of systemic resistance Competition for Iron, nutrient and space	Ongena et al., 2005 Joshi et al., 2012 Lanteigne et al., 2012 Trivedi et al., 2008 Doornbos et al., 2012 Innerebner et al., 2011
Bioremediators	Microorganisms with the ability to sequestration of heavy metals	Siderophores production that Chelate heavy metals	Burd et al., 2000 Ma et al., 2009

varies according to the type of host plant (Garcia et al., 2015). They are also influenced by a number of biotic factors like plant genotypes, plant developmental stages, plant defense mechanisms, other members of the microbial community and abiotic factors like soil composition, soil management and climatic conditions (Vacheron et al., 2013).

7.5.1 PGPR as biofertilizers

Next to water and temperature, nutrients are the environmental factor that most strongly constrains terrestrial plant growth. PGPR promote the plant growth by increasing the availability or uptake of nutrients from a confined nutrient pool in the soil/rhizosphere.

7.5.1.1 Biological nitrogen fixation (BNF)

Nitrogen is the main limiting nutrient for plant growth (Munees & Mulugeta, 2014; Aerts and Chapin, 1999). It is the fourth important element of plant dry mass. Nitrogen is an essential constituent of nucleotides, membrane lipids and amino acids (enzymatic and structural proteins) (Marschner, 1995). The most part of this element is in gaseous form (N_2) inaccessible to animals and plants (Pujic & Normand, 2009). BNF is a major source of nitrogen for plants as a part of environment friendly agricultural practices. According to an estimate, global contribution of biological nitrogen fixation is 180×10^6 metric tons per year. Of this contribution, 83% comes from symbiotic associations, while the rest part of it is provided by free living or associative systems (Graham, 1988). PGPR have the ability to fix atmospheric nitrogen and made it available to plants. Symbiotic nitrogen fixation is a mutualistic relationship between a microbe and the plant. Symbiotic bacteria which act as PGPR are *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, and *Mesorhizobium* with leguminous plants, *Frankia* with non-leguminous trees and shrubs (Zahran, 2001). Non-symbiotic nitrogen fixation is carried out by free living diazotrophs. They stimulate the growth of non-leguminous plants as radish and rice. Non-symbiotic Nitrogen fixing rhizospheric bacteria belongs to genera including *Azoarcus*, *Azotobacter*, *Acetobacter*, *Azospirillum*, *Burkholderia*, *Diazotrophicus*, *Enterobacter*, *Gluconacetobacter*, *Pseudomonas* and cyanobacteria (*Anabaena*, *Nostoc*) (Bhattacharyya and Jha, 2012; Vessey, 2003). Inoculation of biological N_2 -fixing PGPR on crops and crop fields revitalizes growth promoting activity, disease management, and maintains the nitrogen level in agricultural soil (Damam et al., 2016).

7.5.1.2 Phosphate solubilization

Phosphorus is the most important key element in the nutrition of plants, next to nitrogen. It plays an important role in almost all major metabolic processes, including energy transfer, signal transduction, respiration, macromolecular biosynthesis, and photosynthesis (Anand et al., 2016). It is abundantly available in soils in both organic and inorganic forms, but because 95–99% phosphate present in the insoluble, immobilized, and precipitated form. Therefore, it is difficult for plants to absorb it. Plants absorb phosphate only as monobasic (H_2PO_4^-) and dibasic (HPO_4^{2-}) ions. Solubilization and mineralization of phosphorus by phosphate-solubilizing bacteria is an important trait that can be achieved by PGPR. These bacteria secrete different types of organic acids (e.g., carboxylic acid), which lowers the pH in the rhizosphere and thus release the bound forms of phosphate like $\text{Ca}_3(\text{PO}_4)_2$ in the calcareous soils (Sharma et al., 2013). Apart from providing the availability of accumulated phosphate (by solubilization), phosphorus biofertilizers also help in increasing the efficiency of biological nitrogen fixation and render availability of other trace elements such as Fe, Zn, etc., through production of plant growth promoting substances. Phosphate solubilizing PGPR are included in the genera *Arthrobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Microbacterium*, *Pseudomonas*, *Erwinia*, *Rhizobium*, *Mesorhizobium*, *Flavobacterium*, *Rhodococcus*, and *Serratia* and have attracted the attention of agriculturists as soil inoculate improve plant growth and yield (Oteino et al., 2015). Among the heterogenous and naturally abundant microbes of rhizosphere, phosphate solubilizing bacteria (PSB) have provided an alternative biotechnological solution in sustainable agriculture to meet the P demands of plants.

7.5.1.3 Potassium solubilization

Potassium (K) is the third major essential plant nutrient and plays an essential role for enzyme activation, protein synthesis and photosynthesis. As more than 90% of potassium exists in the form of insoluble rock and silicate minerals, the concentration of soluble potassium is usually very low in soil (Parmar and Sindhu, 2013). Potassium deficiency has become a major constraint in crop production (Nath et al., 2017; Meena et al., 2017). Without adequate potassium, plants have poorly developed roots, low seed production, slow growth rate, and a lower yield. It is essential to find an alternative endemic source of potassium for maintaining potassium status and plant uptake in soils for sustaining crop production

(Kumar and Dubey, 2012). The ability of PGPR to solubilize potassium rock by producing and secreting organic acids has been widely investigated (Meena et al., 2014; Shrivastava et al., 2016; Sindhu et al., 2016; Bahadur et al., 2017). PGPR, such as *Acidithiobacillus* sp., *Bacillus edaphicus*, *Ferrooxidans* sp., *Bacillus mucilaginosus*, *Pseudomonas* sp., *Burkholderia* sp., and *Paenibacillus* sp., have been reported to release potassium in accessible form from potassium-bearing minerals in soils (Liu et al., 2012). Therefore, applying potassium solubilizing PGPR as biofertilizer to improve agriculture can reduce the use of agrochemicals and support sustainable crop production (Setiawati and Mutmainnah, 2016; Basak et al., 2017; Wei et al., 2017; Bakhshandeh et al., 2017).

7.5.1.4 Siderophore production (Iron chelation)

Iron is essential for plant growth and development and is required as a cofactor for proteins that are involved in a number of important metabolic processes including photosynthesis and respiration. Iron is the fourth most abundant element in the earth's crust. Unfortunately, this huge quantity of iron is in the ferric ions form (Fe^{3+}) very little assimilated by living organisms (Ammari and Mengel, 2006). To overcome this difficulty and provide iron to the plant, PGPR have developed various iron uptake strategies to survive and to adapt to their environment. One of these strategies is the production of siderophores. Siderophores are small organic molecules produced by microorganisms under iron-limiting conditions that enhance iron uptake capacity (Whipps, 2001; Li et al., 2016). *Pseudomonas* sp., as PGPR, utilizes the siderophores produced by other microbes present in the rhizosphere for fulfilling their ions requirement. More specifically, *Pseudomonas putida* utilize heterologous siderophores produced by other microorganisms to enhance the level of iron available in the natural habitat (Rathore, 2014). According to the chemical function involved in the iron chelation, the siderophores are classified into three classes: phenol catechol, hydroxamate and hydroxycarboxylic acid. Today, more than 500 siderophores are known and the chemical structures of 270 of them were determined (Hider and Kong, 2010). A potent siderophore, such as the ferric-siderophore complex, plays an important role in iron uptake by plants in the presence of other metals, such as nickel and cadmium (Beneduzi et al., 2012). As PGPR can produce siderophores, they are a major asset providing the plant with the required amount of iron. Research regarding the ability of siderophores

to increase iron uptake capacity of plants is however very limited, and considerable research are further required in the context.

7.5.1.5 Zinc solubilization

Zinc is one of the imperative micronutrients required relatively in small concentrations ($5\text{--}100\text{ mg kg}^{-1}$) in tissues for healthy growth and reproduction of plants. Zinc deficiency in plants leads to reduced membrane integrity and synthesis of carbohydrates, auxins, nucleotides, cytochromes, and chlorophyll and develops susceptibility to heat stress (Singh et al., 2005). The worldwide prevalence of Zn deficiency in crop is due to low solubility of Zn, rather than low Zn availability in soil (Iqbal et al., 2010; Gontia-Mishra et al., 2016). Zn deficiency is linked to several factors depending on the soil condition such as, at high pH (>7.0) solubility of Zn decreases with increase in pH, high organic matter and bicarbonate content, high magnesium to calcium ratio and high availability of P and Fe (Wissuwa et al., 2006; Li et al., 2016). Commonly application of inorganic zinc partially caters the plant need as 96–99% of applied Zn is converted into different insoluble forms depending upon the soil types and physicochemical reactions within 7 days of application (Saravanan et al., 2004). These details advocate that the soluble form of Zn fertilizers applied to the fields becomes readily insoluble forms that cannot be assimilated by plants, leading to the Zn deficiency in crops. Zinc solubilizer microbes are potential alternate that could cater plant zinc requirement by solubilising the complex zinc in soil. Zinc solubilizing PGPR helps to solubilize the fixed form of Zn and increases uptake of Zn leading to fortification of grains with Zn (Barbagelata and Mallarino, 2013). Since zinc is a limiting factor in sustainable crop production, importance of Zn solubilizers PGPR has an immense in zinc nutrition to plants (Barbagelata and Mallarino, 2013; Gontia-Mishra et al., 2016).

7.5.2 PGPR as phytostimulators

Phytostimulators or plant growth regulators are organic substances, promote, inhibit, or modify growth and development of plants even at low concentrations ($<1\text{ mM}$) (Damam et al., 2016). Ironically, production of these phytostimulators can also be induced by certain microbes, such as PGPR in plants (Table 7.3). PGPRs produce includes indole acetic acid, cytokinins, gibberellins and inhibitors of ethylene production. Phytostimulators, IAA synthesized by PGPRs affect cell enlargement, cell division, root initiation, growth rate, phototropism, geotropisms, apical

Table 7.3 Efficient PGPR strains as phytostimulator producer in numbers of plants

Phytostimulator produced	PGPR	Host	References
IAA	<i>Aeromonas veronii</i>	Rice	Mehnaz et al., 2001
	<i>Agrobacterium sp.</i>	Lettuce	Barazani and Friedman, 1999
	<i>Alcaligenes piechaudii</i>	Lettuce	Barazani and Friedman, 1999
	<i>Azospirillum brasilense</i>	Wheat	Kaushik et al., 2000
	<i>Bradyrhizobium sp.</i>	Radish	Antoun et al., 1998
	<i>Comamonas acidovorans</i>	Lettuce	Barazani and Friedman, 1999
	<i>Enterobacter cloacae</i>	Rice	Mehnaz et al., 2001
	<i>Rhizobium leguminosarum</i>	Radish	Antoun et al., 1998
	<i>Pseudomonas</i>	<i>Cassia tora</i>	Kumar et al., 2015a
	<i>Pseudomonas, Bacillus</i>	Turmeric	Kumar et al., 2016b
Cytokinin	<i>Paenibacillus polymyxa</i>	Wheat	Timmusk et al., 1999
	<i>Pseudomonas fluorescens</i>	Soybean	Garcia de Salamone et al., 2001
	<i>Rhizobium leguminosarum</i>	Rape & lettuce	
Gibberellin	<i>Bacillus sp.</i>	Alder	Noel et al., 1996
			Gutierrez-Manero et al., 2001

dominance, etc. in plants (Ma et al., 2016; Nath et al., 2017). The production of phytostimulators such as IAA, cytokinin, and gibberellins (Kang et al., 2014; Kumar et al., 2014; Saha et al., 2016a,b) or by the activity of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, an enzyme that can lower plant ethylene levels that are typically increased by a wide variety of environmental stresses such as flooding, drought, heavy metals, organic contaminants, pathogen attacks, and salt stress (Chérel et al., 2013; Das and Pradhan, 2016; Saha et al., 2016a,b; Bahadur et al., 2017).

Tryptophan is an amino acid commonly found in root exudates. It is the main precursor molecule for biosynthesis of IAA in bacteria (Etesami et al., 2009). The IAA production ability may allow bacteria to detoxify excess tryptophan/tryptophan analogs that are deleterious to the bacterial cell. Cytokinins stimulate plant cell division and control root development by inhibiting primary root elongation and lateral root formation

and promoting root hair formation (Werner et al., 2003; Riefler et al., 2006) while gibberellins promote the development of stem tissue, root elongation and lateral root extension (Yaxley et al., 2001). Ethylene is a key phytohormone has a wide range of biological activities that can affect plant growth and development. It plays important role in root initiation, inhibits root elongation, promotes fruit ripening, promotes lower wilting, stimulates seed germination, promotes leaf abscission, activates the synthesis of other plant hormones. At low levels, it can promote plant growth in several plant species while it is normally considered as an inhibitor of plant growth and known as a senescence hormone (Pierik et al., 2006). At high concentration, it induces defoliation and other cellular processes that may lead to reduced crop performance (Bhattacharyya and Jha, 2012). The PGPRs associated to produce phyto-stimulators including the genera *Rhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Bacillus*, *Rhizana*, *Pantoea*, *Arthrobacter*, *Pseudomonas*, *Herbaspirillum*, *Enterobacter*, *Brevundimonas* and *Burkholderia* (Yadegari and Mosadeghzad, 2012; Montañez et al., 2012; Kumar et al., 2014).

7.5.3 PGPR as biopesticides

Phytopathogenic microorganisms are a major and chronic threat to sustainable agriculture and ecosystem sustainability. Regular use of chemical pesticides and fungicides has led to environmental concerns and even cause pathogen resistance, forcing constant development of new agents (Fernando et al., 2005). PGPR with biocontrol traits can be considered as an alternative to the high doses of pesticides applied on crops to deter the pathogens and reduce the disease severity. A large number of mechanisms used by PGPR are involved in biocontrol (Table 7.4) such as direct antagonism via production of antibiotics, siderophores, HCN, hydrolytic enzymes (chitinases, proteases, lipases, etc.), or indirect mechanisms in which the biocontrol organisms act as a probiotic by competing with the pathogen for a niche (Lugtenberg and Kamilova 2009). Antibiosis is the most studied and widely found mechanism of biological control agents (Singh et al., 2017b). Among all the PGPRs strains, *Bacillus* and *Pseudomonas* are the two most important genera well studied extensively for antibiosis mechanisms in the disease management practices (Jayaprakashvel and Mathivanan, 2011; Dominguez-Nunez et al., 2016).

Many PGPRs can synthesize anti-fungal metabolites such as antibiotics, fungal cell wall-lysing enzymes, or hydrogen cyanide, which suppress

Table 7.4 Plant growth promoting rhizobacteria (PGPR) as biopesticides/biocontrol agents against various plant diseases

PGPR	Crop	Disease	References
<i>Azospirillum</i> strains SPS2, WBPS1 and Z2-7	Rice	Rice blast	Naureen et al., 2009
<i>Bacillus amyloliquefaciens</i> 937a, <i>Bacillus subtilis</i> 937b, <i>Bacillus pumilus</i> SE34	Tomato	Tomato mottle virus	Murphy et al., 2000
<i>B. amyloliquefaciens</i> strain IN937a, <i>B. pumilus</i> strain SE34, <i>B. subtilis</i> strain IN937b	Cucumber	Cucumber mosaic virus	Zehnder et al., 2000
<i>Bacillus cereus</i> strains B101R, B212R, A068R	Tomato	Foliar diseases	Silva et al., 2004
<i>Bacillus</i> spp. strains BB11, FH17	Bell pepper	Blight of bell pepper	Jiang et al., 2006
<i>B. pumilus</i> strain INR7	Cucumber	Bacterial wilt	Zehnder et al., 2001
<i>B. pumilus</i> strain SE34	Tobacco	Blue mold	Zhang et al., 2002
<i>B. subtilis</i> strain GBO3, <i>B. pumilus</i> strain INR7, <i>B. pumilus</i> strain T4	Pearl millet	Downy mildew	Niranjan et al., 2003
<i>B. subtilis</i> strain ME488	Cucumber, Pepper	Soilborne pathogens	Chung et al., 2008
<i>Burkholderia</i> strains MBf 21 and MBf15	Maize	Maize rot	Hernandez-Rodriguez et al., 2008
<i>Enterobacter</i> sp.	Chickpea	Fusarium avenaceum	Hynes et al., 2008
<i>Paenibacillus polymyxa</i> strain E681	Sesame	Fungal disease	Ryu et al., 2006
<i>Pseudomonas</i> sp.	White clover Medicago	Acyrtosiphon Kondoi	Kempster et al., 2002
<i>Pseudomonas fluorescens</i> strain CHAO p chitin bioformulations	Banana	Banana bunchy top virus	Kavino et al., 2010
<i>Streptomyces marcescens</i> strain 90–116	Tobacco	Blue mold	Zhang et al., 2002

the growth of fungal pathogens. PGPR and bacterial endophytes play a vital role in the management of various fungal diseases but one of the major problem faced with biocontrol agents is lack of appropriate delivery system. Thus PGPRs as biocontrol agents can be popularized by doing

genetic modifications which could further contribute to sustainable development of agriculture. Therefore, the selected bacterial strains can be used practically for the development of plant growth promoting or bio-control inoculants, together with other plant growth promoting microbes (Laslo et al., 2012).

7.5.4 PGPR as bioremediators

Bioremediation is a process or technique in which living organisms or their products are used naturally or artificially to remediate/ destroy or immobilize pollutants in the environment (Uqab et al., 2016). Bioremediation is recognized as an important tool to restore contaminated sites, reforest eroded areas and degraded ecosystems. Many PGPR are capable of degrading toxic compounds including herbicides, pesticides, solvents, organic compounds and might provide a reasonable and effective measure of disposing toxic compounds (Murali and Mehar, 2014). PGPR capable of producing siderophores that chelate iron as well as other heavy metals like cadmium, lead, nickel, arsenic, aluminium, magnesium, zinc, copper, cobalt and strontium, that can help in adsorbing/absorbing heavy metals from the soil, besides plant growth promotion and phytopathogen suppression (Burd et al., 2000; Ma et al., 2009). At present, studies using PGPR as tools for rhizoremediation are restricted to a few microbial species, such as *Pseudomonas aeruginosa*, genetically engineered *Pseudomonas fluorescens*, and certain *Bacillus* species (Kuiper et al., 2001). Further exploration of PGPR and their application as bioremediators is needed for large scale removal of pollutants in forms of heavy metals or other impurities from soil and water sources. Some notable PGPR strains used in bioremediation are listed in Table 7.5.

7.5.5 PGPR for stress management

Stress is defined as any factor that has a negative effect on plant growth (Foyer et al., 2016). Plant growth is influenced by a variety of stresses due to the soil environment, which is a major constraint for sustainable agricultural production. These stresses can be classified into two groups, biotic and abiotic. Abiotic stress is the primary cause of crop loss, worldwide by more than 30%. Aridity stress imparted by drought, salinity, and high temperature is the most dominant abiotic stress limiting plant growth and productivity (Vejan et al., 2016). The use of PGPR in plant abiotic stress management has been comprehensively studied through bacterial

Table 7.5 Soil bioremediation of heavy metals using PGPR interaction

PGPR	Crop	Heavy metal	References
<i>Achromobacter xylosoxidans</i> Ax10	<i>Brassica juncea</i>	Cu	Ma et al., 2009
<i>Azotobacter chroococcum</i> HKN-5, <i>Bacillus megaterium</i> HKP-1, <i>Bacillus mucilaginosus</i> HKK-1	<i>B. juncea</i>	Pb and Zn	Wu et al., 2006
<i>Bacillus sp.</i> PSB10	<i>Cicer arietinum</i>	Cr	Wani and Khan, 2010
<i>Bacillus subtilis</i> SJ-101	<i>B. juncea</i>	Ni	Zaidi et al., 2006
<i>Bradyrhizobium sp.</i> RM8	<i>Brassica napus</i>	Ni and Zn	Wani et al., 2007
<i>Brevibacillus</i> B-1	<i>Trifolium repens</i>	Zn	Vivas et al., 2006
<i>Kluyvera ascorbata</i> SUD 165, SUD 165/26	<i>B. juncea</i> , <i>Lycopersicon esculentum</i>	Ni, Pb and Zn	Burd et al., 2000
<i>Mesorhizobium huakuii</i> B3	<i>Astragalus sinicus</i>	Cd	Sriprang et al., 2003
<i>Pseudomonas sp.</i> M6, <i>Pseudomonas jessenii</i> M15	<i>Ricinus communis</i>	Ni, Cu and Zn	Rajkumar and Freitas, 2008
<i>Pseudomonas sp.</i> SRA 2, SRA 1, <i>B. cereus</i> SRA 10	<i>B. juncea</i> , <i>Brassica oxyrrhina</i>	Ni	Ma et al., 2009
<i>Serratia sp.</i> SY5	<i>Zea mays</i>	Cd and Cu	Koo and Cho, 2009

strains, such as *Pseudomonas putida* and *Pseudomonas fluorescens* that neutralize the toxic effect of cadmium pollution on barley plants due to their ability to scavenge cadmium ions from soil (Baharlouei et al., 2011). Moreover, improved leaf water status, particularly under salinity and other abiotic stress conditions, has also been reported as an effect of PGPR (Ahmad et al., 2013; Naveed et al., 2014). Biotic stress is caused by different pathogens, such as bacteria, viruses, fungi, nematodes, protists, insects, and viroids, and results in a significant reduction in agricultural yield (Haggag et al., 2015). Biotic stress has adverse impacts on plants, including co-evolution, population dynamics, ecosystem nutrient cycling,

natural habitat ecology, and horticultural plant health (Gusain et al., 2015). Such problems could be solved by using PGPR, such as *Paenibacillus polymyxa* strains B2, B3, B4, *Bacillus amyloliquefaciens* strain HYD-B17, *B. licheniformis* strain HYTAPB18, *B. thuringiensis* strain HYDGRFB19, *P. favisporus* strain BKB30, and *B. subtilis* strain RMPB44. Plants inoculated by soaking their roots or seeds overnight in cultures of PGPR exhibit enormous resistance to different forms of biotic stress (Ngumbi and Kloepper, 2016).



7.6 FUTURE PERSPECTIVE AND CHALLENGES

Keeping in mind the beneficial services rendered by the PGPR (in terms of biofertilization, biopesticide, phyto-stimulator and bioremediation) which exhibit positive influence on crop productivity and agriculture sustainability; we should encourage their successful implementation in the main agriculture system. With better research and development, PGPR use will become a reality and instrumental to fundamental processes that drive sustainability and productivity of agro-ecosystems, thus leading us towards an ideal agricultural system which is sustainable, maintains and improves human health, benefits environment and produces enough food for the increasing world population. And last but not the least, search for new strains of PGPR for biofertilizer and development of microbial diversity map for any region just like nutrient mapping may be helpful too. Advance simulation models related to nature of microbes and their behavioral patterns under changing edapho-climatic conditions may also be developed with suitable technical calibrations and testing for better development and maintenance of agricultural sustainability as well as microbial diversity in near future.

PGPR has been enhancing the agriculture productivity through different mechanisms and processes. For this reason, there is urgent need for application of modern tools and techniques for enhancement of PGPR can serve as key in sustainable agriculture by improving soil fertility, plant tolerance, crop productivity, and maintaining a balanced nutrient cycling. Further studies on selecting suitable rhizosphere microbes and producing microbial communities along with exploring multidisciplinary research that combines applications in biotechnology, nanotechnology, agro

biotechnology, chemical engineering and material science and bringing together different ecological and functional biological approaches can provide new formulations and opportunities with immense potential. Developing suitable alternate formulations viz., liquid inoculants/granular, PGPRs selected for biocontrol against multiple plant pathogens in bioassays are pre-requisite for the advancement of agriculture sustainability.



7.7 CONCLUDING REMARKS

Keeping in view the above-given facts on the different beneficial effects of PGPR, it is amply clear that the use of these PGPR is an attractive as well as economic approach for sustainable agriculture. There is need to create awareness among the farmers about the potential benefits that could be obtained using these PGPR rather than focusing on cost ineffective approaches based on the use of chemical fertilizers. The commercialization of PGPR as biofertilizers should be emphasized. Worldwide, considerable progress has been achieved in the area of PGPR biofertilizer technology. It has been also proved that PGPR are very effective and potential microbes for enriching the crop productivity and soil fertility. PGPR are excellent model systems which can provide the biotechnologist with novel genetic constituents and bioactive chemicals having diverse uses in agriculture and environmental sustainability. As a general conclusion, we can say that many benefits have been reached with the application of microbial biotechnology in agriculture but many challenges as well as opportunities need to be explored for the future sustainable agricultural developments.

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REFERENCES

- Aerts, R., Chapin, F.S., 1999. The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. *Adv. Ecol. Res.* 30, 1–67.
- Ahemad, M., 2012. Implications of bacterial resistance against heavy metals in bioremediation: a review. *Journal of Institute of Integrative Omics and Applied Biotechnology (IIOAB)* 3 (3), 39–46.

- Ahemad, M., Khan, M.S., 2012. Comparative toxicity of selected insecticides to pea plants and growth promotion in response to insecticide-tolerant and plant growth promoting *Rhizobium leguminosarum*. *Crop Protection* 29 (4), 325–329.
- Ahemad, M., Malik, A., 2011. Bioaccumulation of heavy metals by zinc resistant bacteria isolated from agricultural soils irrigated with wastewater. *Bacteriol J* 2, 12–21.
- Ahmad, M., Nadeem, S.M., Naveed, M., Zahir, Z.A., 2016. Potassium-solubilizing bacteria and their application in agriculture. Potassium solubilizing microorganisms for sustainable agriculture. Springer, India, pp. 293–313. Available from: https://doi.org/10.1007/978-81-322-2776-2_21.
- Ahmad, M., Zahir, Z.A., Khalid, M., Nazli, F., Arshad, M., 2013. Efficacy of *Rhizobium* and *Pseudomonas* strains to improve physiology, ionic balance and quality of mung bean under salt-affected conditions on farmers fields. *Plant Physiol. Biochem.* 63, 170–176.
- Alexandratos, N. and Bruinsma, J. (2012), *World agriculture towards 2030/2050: the 2012 revision* (No. 12-03, p. 4) Rome, FAO: ESA Working paper.
- Altieri, M.A., 2004. Linking ecologists and traditional farmers in the search for sustainable agriculture. *Front. Ecol. Environ.* 2 (1), 35–42.
- Ammari, T., Mengel, K., 2006. Total soluble Fe in soil solutions of chemically different soils. *Geoderma* 136 (3), 876–885.
- Anand, K., Kumari, B., Mallick, M.A., 2016. Phosphate solubilizing microbes: An effective and alternative approach as biofertilizers. *International Journal of Pharmacy and Pharmaceutical Sciences* 8 (2), 37–40.
- Antoun, H. and Kloepper, J.W. (2001), Plant growth promoting rhizobacteria in *Encyclopedia of Genetics*, eds S Brenner, JH, Miller, New York, USA. pp. 1477–1480.
- Antoun, H., Prévost, D., 2005. Ecology of plant growth promoting rhizobacteria. In: Siddiqui, Z.A. (Ed.), *PGPR: Biocontrol and biofertilization*. Springer, Netherlands, pp. 1–38.
- Antoun, H., Beauchamp, C.J., Goussard, N., Chabot, R., Lalande, R., 1998. Potential of *Rhizobium* and *Bradyrhizobium* species as plant growth promoting rhizobacteria on non-legumes: effect on radishes (*Raphanus sativus* L.). *Plant Soil* 204 (1), 57–67.
- Ashrafuzzaman, M., Hossen, F.A., Ismail, M.R., Hoque, M.A., Islam, Z.M., Shahidullah, S.M., et al., 2009. Efficiency of plant growth-promoting rhizobacteria (PGPR) for the enhancement of rice growth. *Afr. J. Biotechnol.* 8 (7), 1247–1252.
- Badri, D.V., Zolla, G., Bakker, M.G., Manter, D.K., Vivanco, J.M., 2013. Potential impact of soil microbiomes on the leaf metabolome and on herbivore feeding behavior. *New Phytol.* 198 (1), 264–273.
- Bahadur, I., Maurya, B.R., Meena, V.S., Saha, M., Kumar, A., Aeron, A., 2017. Mineral release dynamics of tricalcium phosphate and waste muscovite by mineral-solubilizing *Rhizobacteria* isolated from Indo-Gangetic Plain of India. *Geomicrobiol. J.* 34 (5), 454–466.
- Baharlouei, J., Pazira, E. and Solhi, M. (2011), Evaluation of inoculation of plant growth-promoting Rhizobacteria on cadmium uptake by canola and barley, in *Environmental Science and Technology : proceedings of the second IPCBEE conference*, pp. 28–32.
- Bakhshandeh, E., Pirdashti, H., Lendeh, K.S., 2017. Phosphate and potassium-solubilizing bacteria effect on the growth of rice. *Ecological Engineering* 103, 164–169.
- Barazani, O.Z., Friedman, J., 1999. Is IAA the major root growth factor secreted from plant-growth-mediating bacteria?. *J. Chem. Ecol.* 25 (10), 2397–2406.
- Barbagelata, P.A., Mallarino, A.P., 2013. Field correlation of potassium soil test methods based on dried and field-moist soil samples for corn and soybean. *Soil Sci. Soc. Am. J.* 77 (1), 318–327.
- Bardgett, R.D., Mommer, L., De Vries, F.T., 2014. Going underground: root traits as drivers of ecosystem processes. *Trends in Ecology and Evolution* 29 (12), 692–699.

- Basak, B.B., Sarkar, B., Biswas, D.R., Sarkar, S., Sanderson, P., Naidu, R., 2017. Bio-intervention of naturally occurring silicate minerals for alternative source of potassium: challenges and opportunities. *Adv. Agron.* 141, 115–145.
- Beneduzi, A., Ambrosini, A., Passaglia, L.M., 2012. Plant growth-promoting rhizobacteria (PGPR): their potential as antagonists and biocontrol agents. *Genetics and Molecular Biology* 35 (4), 1044–1051.
- Bhattacharyya, P.N., Jha, D.K., 2012. Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. *World Journal of Microbiology and Biotechnology* 28 (4), 1327–1350.
- Burd, G.I., Dixon, D.G., Glick, B.R., 2000. Plant growth-promoting bacteria that decrease heavy metal toxicity in plants. *Can. J. Microbiol.* 46 (3), 237–245.
- Calvo, P., Nelson, L., Kloepper, J.W., 2014. Agricultural uses of plant biostimulants. *Plant Soil* 383 (1–2), 3–41.
- Chérel, I., Lefoulon, C., Boeglin, M., Sentenac, H., 2013. Molecular mechanisms involved in plant adaptation to low K⁺ availability. *J. Exp. Bot.* 65 (3), 833–848.
- Chung, S., Kong, H., Buyer, J.S., Lakshman, D.K., Lydon, J., Kim, S.D., et al., 2008. Isolation and partial characterization of *Bacillus subtilis* ME488 for suppression of soil-borne pathogens of cucumber and pepper. *Appl. Microbiol. Biotechnol.* 80 (1), 115–123.
- Damam, M., Kaloori, K., Gaddam, B., Kausar, R., 2016. Plant growth promoting substances (phytohormones) produced by rhizobacterial strains isolated from the rhizosphere of medicinal plants. *Int. J. Pharm. Sci. Rev. Res.* 37 (1), 130–136.
- Das, I., Pradhan, M., 2016. Potassium-solubilizing microorganisms and their role in enhancing soil fertility and health. Potassium solubilizing microorganisms for sustainable agriculture. Springer, India, pp. 281–291. Available from: <http://dx.doi.org/10.1007/978-81-322-2776-220>.
- Dominguez-Nunez, J.A., Benito, B., Berrocal-Lobo, M., Albanesi, A., 2016. Mycorrhizal fungi: role in the solubilization of potassium. Potassium solubilizing microorganisms for sustainable agriculture. Springer, India, pp. 77–98. Available from: <http://dx.doi.org/10.1007/978-81-322-2776-26>.
- Doornbos, R.F., van Loon, L.C., Bakker, P.A., 2012. Impact of root exudates and plant defense signaling on bacterial communities in the rhizosphere. A review. *Agronomy for Sustainable Development* 32 (1), 227–243.
- Elliott, L.F., Lynch, J.M., 1995. The international workshop on establishment of microbial inocula in soils: cooperative research project on biological resource management of the Organization for Economic Cooperation and Development (OECD). *American Journal of Alternative Agriculture* 10 (2), 50–73.
- Etesami, H.A., Alikhani, H.A., Akbari, A.A., 2009. Evaluation of plant growth hormones production (IAA) ability by Iranian soils rhizobial strains and effects of superior strains application on wheat growth indexes. *World Applied Sciences Journal* 6 (11), 1576–1584.
- Fernando, W.D., Nakkeeran, S., Zhang, Y., 2005. Biosynthesis of antibiotics by PGPR and its relation in biocontrol of plant diseases in *PGPR: Biocontrol and Biofertilization*. Springer, Netherlands, pp. 67–109.
- Fernando deAraujo, F., 2008. Inoculação de sementes com *Bacillus subtilis*, formulado com farinha de ostras e desenvolvimento de milho, soja e algodão. *Ciência e Agrotecnologia* 32 (2), 456–462.
- Figueiredo, M.V., Burity, H.A., Martínez, C.R., Chanway, C.P., 2008. Alleviation of drought stress in the common bean (*Phaseolus vulgaris* L.) by co-inoculation with *Paenibacillus polymyxa* and *Rhizobium tropici*. *Appl. Soil Ecol.* 40 (1), 182–188.

- Foyer, C.H., Rasool, B., Davey, J.W., Hancock, R.D., 2016. Cross-tolerance to biotic and abiotic stresses in plants: a focus on resistance to aphid infestation. *J. Exp. Bot.* 67 (7), 2025–2037.
- Franco-Correa, M., Quintana, A., Duque, C., Suarez, C., Rodríguez, M.X., Barea, J.M., 2010. Evaluation of actinomycete strains for key traits related with plant growth promotion and mycorrhiza helping activities. *Appl. Soil Ecol.* 45 (3), 209–217.
- Garcia, F.P., Menendez, E., Rivas, R., 2015. Role of bacterial bio fertilizers in agriculture and forestry. *AIMS Bioengineering* 2, 183–205.
- Garcia de Salamone, I.E., Hynes, R.K., Nelson, L.M., 2001. Cytokinin production by plant growth promoting rhizobacteria and selected mutants. *Can. J. Microbiol.* 47 (5), 404–411.
- Glick, B.R., 2012. Plant growth-promoting bacteria: mechanisms and applications. *Scientifica* 2012, Article ID963401.
- Glick, B.R., Cheng, Z., Czarny, J., Duan, J., 2007. Promotion of plant growth by ACC deaminase-producing soil bacteria. *European Journal of Plant Pathology* 119 (3), 329–339.
- Gomes, R.C., Semedo, L.T.A.S., Soares, R.M.A., Alviano, C.S., Linhares, L.F., Coelho, R.R.R., 2000. Chitinolytic activity of actinomycetes from a cerrado soil and their potential in biocontrol. *Lett. Appl. Microbiol.* 30 (2), 146–150.
- Gontia-Mishra, I., Sapre, S., Sharma, A., Tiwari, S., 2016. Alleviation of mercury toxicity in wheat by the interaction of mercury-tolerant plant growth-promoting rhizobacteria. *J. Plant Growth Regul.* 35 (4), 1000–1012.
- Graham, P.H. (1988), *Principles and Application of Soil Microbiology*, vol. 40, pp. 322–345.
- Gray, E.J., Smith, D.L., 2005. Intracellular and extracellular PGPR: commonalities and distinctions in the plant–bacterium signaling processes. *Soil Biology and Biochemistry* 37 (3), 395–412.
- Gupta, A., Meyer, J.M., Goel, R., 2002. Development of heavy metal-resistant mutants of phosphate solubilizing *Pseudomonas* sp. NBRI 4014 and their characterization. *Curr. Microbiol.* 45 (5), 323–327.
- Gupta, G., Parihar, S.S., Ahirwar, N.K., Snehi, S.K., Singh, V., 2015. Plant growth promoting rhizobacteria (PGPR): current and future prospects for development of sustainable agriculture. *Journal Microbial and Biochemical Technology* 7 (2), 96–102.
- Gusain, Y.S., Singh, U.S., Sharma, A.K., 2015. Bacterial mediated amelioration of drought stress in drought tolerant and susceptible cultivars of rice (*Oryza sativa* L.). *Afr. J. Biotechnol.* 14 (9), 764–773.
- Gutierrez-Manero, F.J., Ramos-Solano, B., Probanza, A., Mehouchi, J., Tadeo, F.R., Talon, M., 2001. The plant-growth-promoting rhizobacteria *Bacillus pumilus* and *Bacillus licheniformis* produce high amounts of physiologically active gibberellins. *Physiol. Plant.* 111 (2), 206–211.
- Haas, D., Défago, G., 2005. Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nat. Rev. Microbiol.* 3 (4), 307–319. Available from: <https://doi.org/10.1038/nrmicro1129>.
- Haggag, W.M., Abouziena, H.F., Abd-El-Kreem, F., El Habbasha, S., 2015. Agriculture biotechnology for management of multiple biotic and abiotic environmental stress in crops. *J. Chem. Pharm. Res.* 7 (10), 882–889.
- Haghighi, B.J., Alizadeh, O., Firoozabadi, A.H., 2011. The role of plant growth promoting rhizobacteria (PGPR) in sustainable agriculture. *Advances in Environmental Biology* 5 (10), 3079–3083.
- Halder, A.K., Mishra, A.K., Chakrabarty, P.K., 1991. Solubilization of inorganic phosphates by Bradyrhizobium. *Indian J. Exp. Biol.* 29 (1), 28–31.

- Hayat, R., Ali, S., Amara, U., Khalid, R., Ahmed, I., 2010. Soil beneficial bacteria and their role in plant growth promotion: a review. *Annals of Microbiology* 60 (4), 579–598.
- Hernandez-Rodriguez, A., Heydrich-Pérez, M., Acebo-Guerrero, Y., Velazquez-Del Valle, M.G., Hernandez-Lauzardo, A.N., 2008. Antagonistic activity of Cuban native rhizobacteria against *Fusarium verticillioides* (Sacc.) Nirenb. in maize (*Zea mays* L.). *Appl. Soil Ecol.* 39 (2), 180–186.
- Hider, R.C., Kong, X., 2010. Chemistry and biology of siderophores. *Nat. Prod. Rep.* 27 (5), 637–657.
- Hynes, R.K., Leung, G.C., Hirkala, D.L., Nelson, L.M., 2008. Isolation, selection, and characterization of beneficial rhizobacteria from pea, lentil, and chickpea grown in western Canada. *Can. J. Microbiol.* 54 (4), 248–258.
- Innerebner, G., Knief, C., Vorholt, J.A., 2011. Protection of *Arabidopsis thaliana* against leaf-pathogenic *Pseudomonas syringae* by *Sphingomonas* strains in a controlled model system. *Appl. Environ. Microbiol.* 77 (10), 3202–3210.
- Iqbal, U., Jamil, N., Ali, I., Hasnain, S., 2010. Effect of zinc-phosphate-solubilizing bacterial isolates on growth of *Vigna radiata*. *Annals of Microbiology* 60 (2), 243–248.
- Jahanian, A., Chaichi, M.R., Rezaei, K., Rezayazdi, K., Khavazi, K., 2012. The effect of plant growth promoting rhizobacteria (PGPR) on germination and primary growth of artichoke (*Cynara scolymus*). *Journal of Crop Science and Biotechnology* 4, 923–929.
- Jayaprakashvel, M., Mathivanan, N., 2011. Management of plant diseases by microbial metabolites. In: Maheshwari, D.K. (Ed.), *Bacteria in agrobiology: plant nutrient management*. Springer, Berlin, Heidelberg, pp. 237–265. Available from: <http://dx.doi.org/10.1007/978-3-642-21061-710>.
- Jha, B., Gontia, I., Hartmann, A., 2012. The roots of the halophyte *Salicornia brachiata* are a source of new halotolerant diazotrophic bacteria with plant growth-promoting potential. *Plant Soil* 356 (1–2), 265–277.
- Jiang, Z.Q., Guo, Y.H., Li, S.M., Qi, H.Y., Guo, J.H., 2006. Evaluation of biocontrol efficiency of different *Bacillus* preparations and field application methods against *Phytophthora* blight of bell pepper. *Biological control* 36 (2), 216–223.
- Joshi, Y.B., Chu, J., Praticò, D., 2012. Stress hormone leads to memory deficits and altered tau phosphorylation in a model of Alzheimers disease. *Journal of Alzheimers Disease* 31 (1), 167–176.
- Kang, S.M., Joo, G.J., Hamayun, M., Na, C.I., Shin, D.H., Kim, H.Y., et al., 2009. Gibberellin production and phosphate solubilization by newly isolated strain of *Acinetobacter calcoaceticus* and its effect on plant growth. *Biotechnol. Lett.* 31 (2), 277–281.
- Kang, S.M., Khan, A.L., Waqas, M., You, Y.H., Kim, J.H., Kim, J.G., et al., 2014. Plant growth-promoting rhizobacteria reduce adverse effects of salinity and osmotic stress by regulating phytohormones and antioxidants in *Cucumis sativus*. *Journal of Plant Interactions* 9 (1), 673–682.
- Kapulnik, Y., Okon, Y., Kigel, J., Nur, I., Henis, Y., 1981. Effects of temperature, nitrogen fertilization, and plant age on nitrogen fixation by *Setaria italica* inoculated with *Azospirillum brasilense* (strain cd). *Plant Physiol.* 68 (2), 340–343.
- Kaushik, R., Saxena, A., Tilak, K., 2000. Selection of Tn5:lacZ mutants isogenic to wild type *Azospirillum brasilense* strains capable of growing at sub-optimal temperature. *World Journal of Microbiology and Biotechnology* 16 (6), 567–570.
- Kavino, M., Harish, S., Kumar, N., Saravanakumar, D., Samiyappan, R., 2010. Effect of chitinolytic PGPR on growth, yield and physiological attributes of banana (*Musa spp.*) under field conditions. *Appl. Soil Ecol.* 45 (2), 71–77.

- Kempster, V.N., Scott, E.S., Davies, K.A., 2002. Evidence for systemic, cross-resistance in white clover (*Trifolium repens*) and annual medic (*Medicago truncatula* var *truncatula*) induced by biological and chemical agents. *Biocontrol Science and Technology* 12 (5), 615–623.
- Khan, A.G., 2005. Role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation. *J. Trace Elem. Med. Biol.* 18 (4), 355–364.
- Kloepper, J.W., Schroth, M.N., Miller, T.D., 1980. Effects of rhizosphere colonization by plant growth-promoting rhizobacteria on potato plant development and yield. *Phytopathology* 70 (11), 1078–1082.
- Koo, S.Y., Cho, K.S., 2009. Isolation and characterization of a plant growth-promoting rhizobacterium, *Serratia* sp. SY5. *J. Microbiol. Biotechnol.* 19 (11), 1431–1438.
- Kuiper, I., Bloembergen, G.V., Lugtenberg, B.J., 2001. Selection of a plant-bacterium pair as a novel tool for rhizostimulation of polycyclic aromatic hydrocarbon-degrading bacteria. *Mol. Plant-Microbe Interact.* 14 (10), 1197–1205.
- Kumar, A., Kumar, A., Pratush, A., 2014. Molecular diversity and functional variability of environmental isolates of *Bacillus* species. *SpringerPlus* 3 (1), 312.
- Kumar, A., Vandana, R.S., Singh, M., Pandey, K.D., 2015b. Plant growth promoting rhizobacteria (PGPR). A promising approach for disease management. In: Singh, J.S., Singh, D.P. (Eds.), *Microbes and environmental management*. Studium Press, New Delhi, pp. 195–209.
- Kumar, A., Singh, R., Yadav, A., Giri, D.D., Singh, P.K., Pandey, K.D., 2016a. Isolation and characterization of bacterial endophytes of *Curcuma longa* L. *3 Biotech* 6, 60.
- Kumar, A., Singh, V., Singh, M., Singh, P.P., Singh, S.K., Singh, P.K., et al., 2016b. Isolation of plant growth promoting rhizobacteria and their impact on growth and curcumin content in *Curcuma longa* L. *Biocatalysis and Agriculture Biotechnology* 8, 1–7.
- Kumar, A., Verma, H., Singh, V.K., Singh, P.P., Singh, S.K., Ansari, W.A., et al., 2017a. Role of *Pseudomonas* sp. in Sustainable Agriculture and Disease Management. *Agriculturally Important Microbes for Sustainable Agriculture*. Springer, Singapore, pp. 195–215.
- Kumar, A., Maurya, B.R., Raghuwanshi, R., Meena, V.S., Islam, M.T., 2017b. Co-inoculation with *Enterobacter* and *Rhizobacteria* on yield and nutrient uptake by wheat (*Triticum aestivum* L.) in the Alluvial soil under Indo-Gangetic plain of India. *J. Plant Growth Regul.* 1–10. Available from: <https://doi.org/10.1007/s00344-016-9663-5>.
- Kumar, P., Dubey, R.C., 2012. Plant growth promoting rhizobacteria for biocontrol of phytopathogens and yield enhancement of *Phaseolus vulgaris* L. *Journal of Current Perspectives in Applied Microbiology* 1, 6–38.
- Kumar, V., Kumar, A., Pandey, K.D., Roy, B.K., 2015a. Isolation and characterization of bacterial endophytes from the roots of *Cassia tora* L. *Annals of Microbiology* 65, 1391–1399.
- Lanteigne, C., Gadkar, V.J., Wallon, T., Novinscak, A., Filion, M., 2012. Production of DAPG and HCN by *Pseudomonas* sp. LBUM300 contributes to the biological control of bacterial canker of tomato. *Phytopathology* 102 (10), 967–973.
- Laslo, E., Gyorgy, E., Mara, G., Tamas, E., Abraham, B., Lanyi, S., 2012. Screening of plant growth promoting rhizobacteria as potential microbial inoculants. *Crop Protection* 40, 43–48.
- Li, M., Ahammed, G.J., Li, C., Bao, X., Yu, J., Huang, C., et al., 2016. Brassinosteroid ameliorates zinc oxide nanoparticles-induced oxidative stress by improving antioxidant potential and redox homeostasis in tomato seedling. *Frontiers in plant science* 7, 615.
- Liu, D., Lian, B., Dong, H., 2012. Isolation of *Paenibacillus* sp. and assessment of its potential for enhancing mineral weathering. *Geomicrobiol. J.* 29 (5), 413–421.

- Lugtenberg, B., Kamilova, F., 2009. Plant-growth-promoting rhizobacteria. *Annu. Rev. Microbiol.* 63, 541–556.
- Ma, Y., Rajkumar, M., Freitas, H., 2009. Improvement of plant growth and nickel uptake by nickel resistant-plant-growth promoting bacteria. *J. Hazard. Mater.* 166 (2), 1154–1161.
- Ma, Y., Rajkumar, M., Luo, Y., Freitas, H., 2011. Inoculation of endophytic bacteria on host and non-host plants-effects on plant growth and Ni uptake. *J. Hazard. Mater.* 195, 230–237.
- Ma, Y., Rajkumar, M., Zhang, C., Freitas, H., 2016. Beneficial role of bacterial endophytes in heavy metal phytoremediation. *J. Environ. Manage.* 174, 14–25.
- Mahdi, S.S., Hassan, G.I., Samoon, S.A., Rather, H.A., Dar, S.A., Zehra, B., 2010. Bio-fertilizers in organic agriculture. *Journal of Phytology* 2 (10), 42–54.
- Marschner, H., 1995. Mineral nutrition of higher plants. Academic Press, London.
- Martínez-Viveros, O., Jorquera, M.A., Crowley, D.E., Gajardo, G.M.L.M., Mora, M.L., 2010. Mechanisms and practical considerations involved in plant growth promotion by rhizobacteria. *Journal of Soil Science and Plant Nutrition* 10 (3), 293–319.
- Mayak, S., Tirosh, T., Glick, B.R., 2004. Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. *Plant Physiol. Biochem.* 42 (6), 565–572.
- Meena, V.S., Maurya, B.R., Verma, J.P., 2014. Does a rhizospheric microorganism enhance K^+ availability in agricultural soils?. *Microbiol. Res.* 169 (5), 337–347.
- Meena, V.S., Mishra, P.K., Bisht, J.K., Pattanayak, A., 2017. *Agriculturally Important Microbes for Sustainable Agriculture, Volume I: Plant-soil-microbe nexus*. Springer, Singapore. Available from: <http://dx.doi.org/10.1007/978-981-10-5589-8>.
- Mehnaz, S., Mirza, M.S., Haurat, J., Bally, R., Normand, P., Bano, A., et al., 2001. Isolation and 16S rRNA sequence analysis of the beneficial bacteria from the rhizosphere of rice. *Can. J. Microbiol.* 47 (2), 110–117.
- Mendes, R., Kruijt, M., de Bruijn, I., Dekkers, E., van der Voort, M., Schneider, J.H., et al., 2011. Deciphering the rhizosphere microbiome for disease-suppressive bacteria. *Science* 332 (6033), 1097–1100.
- Merzaeva, O.V., Shirokikh, I.G., 2006. Colonization of plant rhizosphere by actinomycetes of different genera. *Microbiology* 75 (2), 226–230.
- Montañez, A., Blanco, A.R., Barlocco, C., Beracochea, M., Sicardi, M., 2012. Characterization of cultivable putative endophytic plant growth promoting bacteria associated with maize cultivars (*Zea mays* L.) and their inoculation effects in vitro. *Appl. Soil Ecol.* 58, 21–28.
- Morrissey, J.P., Dow, J.M., Mark, G.L., Gara, F.O., 2004. Are microbes at the root of a solution to world food production? *EMBO Rep.* 5 (10), 922–926. Available from: <https://doi.org/10.1038/sj.embor.7400263>. PMID: 15459741.
- Murali, O., Mehar, S.K., 2014. Bioremediation of heavy metals using *Spirulina*. *International Journal of Geology, Earth and Environmental Sciences* 4 (1), 244–249.
- Murphy, J.F., Zehnder, G.W., Schuster, D.J., Sikora, E.J., Polston, J.E., Kloepper, J.W., 2000. Plant growth-promoting rhizobacterial mediated protection in tomato against Tomato mottle virus. *Plant Disease* 84 (7), 779–784.
- Nath, D., Maurya, B.R., Meena, V.S., 2017. Documentation of five potassium-and phosphorus-solubilizing bacteria for their K and P-solubilization ability from various minerals. *Biocatalysis and Agricultural Biotechnology* 10, 174–181.
- Naureen, Z., Price, A.H., Hafeez, F.Y., Roberts, M.R., 2009. Identification of rice blast disease-suppressing bacterial strains from the rhizosphere of rice grown in Pakistan. *Crop Protection* 28 (12), 1052–1060.
- Naveed, M., Hussain, M.B., Zahir, Z.A., Mitter, B., Sessitsch, A., 2014. Drought stress amelioration in wheat through inoculation with *Burkholderia phytofirmans* strain PsjN. *Plant Growth Regul.* 73 (2), 121–131.

- Ngumbi, E., Kloepper, J., 2016. Bacterial-mediated drought tolerance: current and future prospects. *Appl. Soil Ecol.* 105, 109–125.
- Noel, T.C., Sheng, C., Yost, C.K., Pharis, R.P., Hynes, M.F., 1996. *Rhizobium leguminosarum* as a plant growth-promoting rhizobacterium: direct growth promotion of canola and lettuce. *Can. J. Microbiol.* 42 (3), 279–283.
- Nogueira da Silva, V., de Souza Fernandes da Silva, L.E., do Vale Barreto Figueiredo, M., 2006. Atuação de rizóbios com rizobactéria promotora de crescimento em plantas na cultura do caupi (*Vigna unguiculata* [L.] Walp.). *Acta Scientiarum Agronomy* 28 (3), 407–412.
- Ongena, M., Jacques, P., Touré, Y., Destain, J., Jabrane, A., Thonart, P., 2005. Involvement of fengycin-type lipopeptides in the multifaceted biocontrol potential of *Bacillus subtilis*. *Appl. Microbiol. Biotechnol.* 69 (1), 29.
- Ordookhani, K., Zare, M., 2011. Effect of *Pseudomonas*, *Azotobacter* and arbuscular mycorrhiza fungi on lycopene, antioxidant activity and total soluble solid in tomato (*Lycopersicon esculentum* F1 Hybrid, Delba). *Advances in Environmental Biology* 5, 1290–1295.
- Oteino, N., Lally, R.D., Kiwanuka, S., Lloyd, A., Ryan, D., Germaine, K.J., et al., 2015. Plant growth promotion induced by phosphate solubilizing endophytic *Pseudomonas* isolates. *Front. Microbiol.* 6, 745.
- Parmar, P., Sindhu, S.S., 2013. Potassium solubilization by rhizosphere bacteria: influence of nutritional and environmental conditions. *Journal of Microbiology Research* 3 (1), 25–31.
- Patil, S.S., Adetutu, E.M., Rochow, J., Mitchell, J.G., Ball, A.S., 2014. Sustainable remediation: electrochemically assisted microbial dechlorination of tetrachloroethene-contaminated groundwater. *Microbial Biotechnology* 7 (1), 54–63.
- Paustian, K., Lehmann, J., Ogle, S., Reay, D., Robertson, G.P., Smith, P., 2016. Climate-smart soils. *Nature* 532 (7597), 49–57.
- Pierik, R., Tholen, D., Poorter, H., Visser, E.J., Voesenek, L.A., 2006. The Janus face of ethylene: growth inhibition and stimulation. *Trends Plant Sci.* 11 (4), 176–183.
- Rajkumar, M., Freitas, H., 2008. Influence of metal resistant-plant growth-promoting bacteria on the growth of *Ricinus communis* in soil contaminated with heavy metals. *Chemosphere* 71 (5), 834–842.
- Rathore, P., 2014. A review on approaches to develop plant growth promoting rhizobacteria. *International Journal of Recent Scientific Research* 5, 403–407.
- Riefler, M., Novak, O., Strnad, M., Schmölling, T., 2006. *Arabidopsis* cytokinin receptor mutants reveal functions in shoot growth, leaf senescence, seed size, germination, root development, and cytokinin metabolism. *The Plant Cell* 18 (1), 40–54.
- Russo, A., Vettori, L., Felici, C., Fiaschi, G., Morini, S., Toffanin, A., 2008. Enhanced micropropagation response and biocontrol effect of *Azospirillum brasilense* Sp245 on *Prunus cerasifera* L. clone Mr. S 2/5 plants. *J. Biotechnol.* 134 (3), 312–319.
- Ryu, C.M., Kim, J., Choi, O., Kim, S.H., Park, C.S., 2006. Improvement of biological control capacity of *Paenibacillus polymyxa* E681 by seed pelleting on sesame. *Biological Control* 39 (3), 282–289.
- Saha, M., Maurya, B.R., Bahadur, I., Kumar, A., Meena, V.S., 2016a. Can Potassium-Solubilizing Bacteria Mitigate the Potassium Problems in India?. *Potassium solubilizing microorganisms for sustainable agriculture*. Springer, India, pp. 127–136.
- Saha, M., Maurya, B.R., Meena, V.S., Bahadur, I., Kumar, A., 2016b. Identification and characterization of potassium solubilizing bacteria (KSB) from Indo-Gangetic Plains of India. *Biocatalysis and Agricultural Biotechnology* 7, 202–209.
- Saharan, B.S., Nehra, V., 2011. Plant growth promoting rhizobacteria: a critical review. *International Journal of Life Science and Medical Research* 21 (1), 30.

- Sandhya, V.Z.A.S., Grover, M., Reddy, G., Venkateswarlu, B., 2009. Alleviation of drought stress effects in sunflower seedlings by the exopolysaccharides producing *Pseudomonas putida* strain GAP-P45. *Biology and fertility of soils* 46 (1), 17–26.
- Santiago de Freitas, A.D., Lucena Vieira, C., de Rosália e Silva Santos, C.E., Pereira Stamford, N., do Carmo Catanho Pereira de Lyra, M., 2007. Caracterização de rizóbios isolados de Jacatupé cultivado em solo salino do estado de Pernambuco, Brasil. *Bragantia* 66 (3), 497–504.
- Saravanan, V.S., Subramoniam, S.R., Raj, S.A., 2004. Assessing in vitro solubilization potential of different zinc solubilizing bacterial (zsb) isolates. *Brazilian Journal of Microbiology* 35 (1–2), 121–125.
- Sarkar, D., Meena V.S., Haldar, A., Rakshit, R. (2017), Site-specific nutrient management (SSNM): a unique approach towards maintaining soil health in *The adaptive soil management: from theory to practices*, pp. 69–88. Available from: doi:10.1007/978-981-10-3638-5-3.
- Setiawati, T.C., Mutmainnah, L., 2016. Solubilization of potassium containing mineral by microorganisms from sugarcane rhizosphere. *Agric. Agric. Sci. Procedia* 9, 108–117.
- Sgro, V., Cassán, F., Masciarelli, O., Del Papa, M.F., Lagares, A., Luna, V., 2009. Isolation and characterization of endophytic plant growth-promoting (PGPB) or stress homeostasis-regulating (PSHB) bacteria associated to the halophyte *Prosopis strombulifera*. *Appl. Microbiol. Biotechnol.* 85 (2), 371–381.
- Shrivastava, M., Srivastava, P.C., DSouza, S.F., 2016. KSM soil diversity and mineral solubilization, in relation to crop production and molecular mechanism. *Potassium Solubilizing Microorganisms for Sustainable Agriculture*. Springer, India, pp. 221–234.
- Silva, H.S.A., da Silva Romeiro, R., Macagnan, D., de Almeida Halfeld-Vieira, B., Pereira, M.C.B., Mounteer, A., 2004. Rhizobacterial induction of systemic resistance in tomato plants: non-specific protection and increase in enzyme activities. *Biological Control* 29 (2), 288–295.
- Sindhu, S.S., Parmar, P., Phour, M., Sehrawat, A., 2016. Potassium-solubilizing microorganisms (KSMs) and its effect on plant growth improvement. *Potassium Solubilizing Microorganisms for Sustainable Agriculture*. Springer, India, pp. 171–185. Available from: <http://dx.doi.org/10.1007/978-81-322-2776-213>.
- Singh, B., Natesan, S.K.A., Singh, B.K., Usha, K., 2005. Improving zinc efficiency of cereals under zinc deficiency. *Curr. Sci.* 88 (1), 36–44.
- Singh, B.K., Millard, P., Whiteley, A.S., Murrell, J.C., 2004. Unravelling rhizosphere–microbial interactions: opportunities and limitations. *Trends Microbiol.* 12 (8), 386–393.
- Singh, M., Kumar, A., Singh, R., Pandey, K.D., 2017a. Endophytic bacteria: a new source of bioactive compounds. *3 Biotech* 7, 315.
- Singh, V.K., Singh, A.K., Kumar, A., 2017b. Disease management of tomato through PGPB: current trends and future perspective. *3 Biotech* 7 (4), 255.
- Somers, E., Vanderleyden, J., Srinivasan, M., 2004. Rhizosphere bacterial signalling: a love parade beneath our feet. *Crit. Rev. Microbiol.* 30 (4), 205–240.
- Sousa, C.S., Soares, A.C.F., Garrido, M.S., 2008. Characterization of streptomycetes with potential to promote plant growth and biocontrol. *Scientia Agricola* 65 (1), 50–55.
- Sriprang, R., Hayashi, M., Ono, H., Takagi, M., Hirata, K., Murooka, Y., 2003. Enhanced accumulation of Cd²⁺ by a *Mesorhizobium* sp. transformed with a gene from *Arabidopsis thaliana* coding for phytochelatin synthase. *Appl. Environ. Microbiol.* 69 (3), 1791–1796.
- Tank, N., Saraf, M., 2010. Salinity-resistant plant growth promoting rhizobacteria ameliorates sodium chloride stress on tomato plants. *J. Plant Interact.* 5 (1), 51–58.

- Terkina, I.A., Parfenova, V.V., Ahn, T.S., 2006. Antagonistic activity of actinomycetes of Lake Baikal. *Applied Biochemistry and Microbiology* 42 (2), 173–176.
- Tian, F., Ding, Y., Zhu, H., Yao, L., Du, B., 2009. Genetic diversity of siderophore-producing bacteria of tobacco rhizosphere. *Brazil. Journ. Micro.* 40 (2), 276–284.
- Timmusk, S., Nicander, B., Granhall, U., Tillberg, E., 1999. Cytokinin production by *Paenibacillus polymyxa*. *Soil Biology and Biochemistry* 31 (13), 1847–1852.
- Uqab, B., Mudasir, S., Nazir, R., 2016. Review on bioremediation of pesticides. *Journal of Bioremediation and Biodegradation* 7 (3), 343.
- Vacheron, J., Desbrosses, G., Bouffaud, M.L., Touraine, B., Moëgne-Loccoz, Y., Muller, D., et al., 2013. Plant growth-promoting rhizobacteria and root system functioning. *Frontiers in Plant Science* 4 (356), 1–19.
- Vansuyt, G., Robin, A., Briat, J.F., Curie, C., Lemanceau, P., 2007. Iron acquisition from Fe-pyoverdine by *Arabidopsis thaliana*. *Mol. Plant-Microbe Interact.* 20 (4), 441–447.
- Vaxevanidou, K., Christou, C., Kremmydas, G.F., Georgakopoulos, D.G., Papassiopi, N., 2015. Role of indigenous arsenate and iron (III) Respiring microorganisms in controlling the mobilization of arsenic in a contaminated soil Sample. *Bull. Environ. Contam. Toxicol.* 94 (3), 282–288.
- Vejan, P., Abdullah, R., Khadiran, T., Ismail, S., Nasrulhaq Boyce, A., 2016. Role of plant growth promoting rhizobacteria in agricultural sustainability-a review. *Molecules* 21 (5), 573.
- Verma, J.P., Yadav, J., Tiwari, K.N., Lavakush, S.V., 2010. Impact of plant growth promoting rhizobacteria on crop production. *International Journal of Agricultural Research* 5 (11), 954–983.
- Verma, R., Maurya, B.R., Meena, V.S., Dotaniya, M.L., Deewan, P., Jajoria, M., 2017. Enhancing production potential of cabbage and improves soil fertility status of Indo-Gangetic Plain through application of bio-organics and mineral fertilizer. *International Journal of Current Microbiology and Applied Sciences* 6 (3), 301–309.
- Vessey, J.K., 2003. Plant growth promoting rhizobacteria as biofertilizers. *Plant Soil* 255 (2), 571–586.
- Vivas, A., Biro, B., Ruiz-Lozano, J.M., Barea, J.M., Azcon, R., 2006. Two bacterial strains isolated from a Zn-polluted soil enhance plant growth and mycorrhizal efficiency under Zn-toxicity. *Chemosphere* 62 (9), 1523–1533.
- Wani, P.A., Khan, M.S., 2010. *Bacillus* species enhance growth parameters of chickpea (*Cicer arietinum* L.) in chromium stressed soils. *Food Chem. Toxicol.* 48 (11), 3262–3267.
- Wani, P.A., Khan, M.S., Zaidi, A., 2007. Effect of metal tolerant plant growth promoting *Bradyrhizobium* sp. (vigna) on growth, symbiosis, seed yield and metal uptake by greengram plants. *Chemosphere* 70 (1), 36–45.
- Wei, Y., Zhao, Y., Fan, Y., Lu, Q., Li, M., Wei, Q., et al., 2017. Impact of phosphate-solubilizing bacteria inoculation methods on phosphorus transformation and long-term utilization in composting. *Bioresour. Technol.* 241, 134–141.
- Werner, D., 2000. Organic signals between plants and microorganisms. In: Pinton, R., Varanini, Z., Nannipieri, P. (Eds.), *The Rhizosphere: Biochemistry and Organic Substances at the Soil–Plant Interface*. Marcel Dekker, New York, USA, pp. 197–222.
- Werner, T., Motyka, V., Laucou, V., Smets, R., Van Onckelen, H., Schmulling, T., 2003. Cytokinin-deficient transgenic *Arabidopsis* plants show multiple developmental alterations indicating opposite functions of cytokinins in the regulation of shoot and root meristem activity. *The Plant Cell* 15 (11), 2532–2550.
- Weyens, N., Truyens, S., Dupae, J., Newman, L., Van der Lelie, D., Carleer, R., et al., 2010. Potential of *Pseudomonas putida* W619-TCE to reduce TCE phytotoxicity and evapotranspiration in poplar cuttings. *Environmental Pollution* 158 (9), 2915–2919.

- Whipps, J.M., 2001. Microbial interactions and biocontrol in the rhizosphere. *J. Exp. Bot.* 52 (suppl 1), 487–511.
- Wissuwa, M., Ismail, A.M., Yanagihara, S., 2006. Effects of zinc deficiency on rice growth and genetic factors contributing to tolerance. *Plant Physiol.* 142 (2), 731–741.
- Wood, N.T., 2001. Nodulation by numbers: the role of ethylene in symbiotic nitrogen fixation. *Trends Plant Sci.* 6 (11), 501–502.
- Wu, C.H., Wood, T.K., Mulchandani, A., Chen, W., 2006. Engineering plant-microbe symbiosis for rhizoremediation of heavy metals. *Appl. Environ. Microbiol.* 72 (2), 1129–1134.
- Wu, S.C., Cao, Z.H., Li, Z.G., Cheung, K.C., Wong, M.H., 2005. Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. *Geoderma* 125 (1), 155–166.
- Xiang, W., Zhao, L., Xu, X., Qin, Y., Yu, G., 2012. Mutual information flow between beneficial microorganisms and the roots of host plants determined the bio-functions of biofertilizers. *Am. J. Plant Sci.* 3 (8), 1115–1120.
- Yadegari, M., Mosadeghzad, Z., 2012. Biofertilizers effects on quantitative and qualitative yield of Thyme (*Thymus vulgaris*). *African Journal of Agricultural Research* 7 (34), 4716–4723.
- Yaxley, J.R., Ross, J.J., Sherriff, L.J., Reid, J.B., 2001. Gibberellin biosynthesis mutations and root development in pea. *Plant Physiol.* 125 (2), 627–633.
- Yazdani, M., Bahmanyar, M.A., Pirdashti, H., Esmaili, M.A., 2009. Effect of phosphate solubilization microorganisms (PSM) and plant growth promoting rhizobacteria (PGPR) on yield and yield components of corn (*Zea mays* L.). *World Academy of Science, Engineering and Technology* 37, 90–92.
- Zahedi, H., 2016. Growth-promoting effect of potassium-solubilizing microorganisms on some crop species. In: Meena, V.S., Maurya, B.R., Verma, J.P., Meena, R.S. (Eds.), *Potassium solubilizing microorganisms for sustainable agriculture*. Springer, India, pp. 31–42. Available from: <https://doi.org/10.1007/978-81-322-2776-2-3>.
- Zahran, H.H., 2001. Rhizobia from wild legumes: diversity, taxonomy, ecology, nitrogen fixation and biotechnology. *J. Biotechnol.* 91 (2), 143–153.
- Zaidi, S., Usmani, S., Singh, B.R., Musarrat, J., 2006. Significance of *Bacillus subtilis* strain SJ-101 as a bioinoculant for concurrent plant growth promotion and nickel accumulation in *Brassica juncea*. *Chemosphere* 64 (6), 991–997.
- Zakry, F.A.A., Shamsuddin, Z.H., Rahim, K.A., Zakaria, Z.Z., Rahim, A.A., 2012. Inoculation of *Bacillus sphaericus* UPMB-10 to young oil palm and measurement of its uptake of fixed nitrogen using the ¹⁵N isotope dilution technique. *Microbes and Environments* 27 (3), 257–262.
- Zehnder, G.W., Yao, C., Murphy, J.F., Sikora, E.R., Kloepper, J.W., 2000. Induction of resistance in tomato against cucumber mosaic cucumovirus by plant growth-promoting rhizobacteria. *Biocontrol* 45 (1), 127–137.
- Zehnder, G.W., Murphy, J.F., Sikora, E.J., Kloepper, J.W., 2001. Application of rhizobacteria for induced resistance. *European Journal of Plant Pathology* 107 (1), 39–50.
- Zhang, S., Moyne, A.L., Reddy, M.S., Kloepper, J.W., 2002. The role of salicylic acid in induced systemic resistance elicited by plant growth-promoting rhizobacteria against blue mold of tobacco. *Biological Control* 25 (3), 288–296.
- Zolla, G., Badri, D.V., Bakker, M.G., Manter, D.K., Vivanco, J.M., 2013. Soil microbiomes vary in their ability to confer drought tolerance to Arabidopsis. *Appl. Soil Ecol.* 68, 1–9.

FURTHER READING

- Ahemad, M., Khan, M., 2011a. Toxicological assessment of selective pesticides towards plant growth promoting activities of phosphate solubilizing *Pseudomonas aeruginosa*. *Acta microbiologica et immunologica Hungarica* 58 (3), 169–187.
- Ahemad, M., Khan, M.S., 2011b. Effects of insecticides on plant-growth-promoting activities of phosphate solubilizing *Rhizobacterium Klebsiella sp.* strain PS19. *Pestic. Biochem. Physiol.* 100 (1), 51–56.
- Liu, W., Wang, Q., Hou, J., Tu, C., Luo, Y., Christie, P., 2016. Whole genome analysis of halotolerant and alkalotolerant plant growth-promoting rhizobacterium *Klebsiella sp.* D5A. *Sci. Rep.* 6, 26710.
- Raj, S.N., Deepak, S.A., Basavaraju, P., Shetty, H.S., Reddy, M.S., Kloepper, J.W., 2003. Comparative performance of formulations of plant growth promoting rhizobacteria in growth promotion and suppression of downy mildew in pearl millet. *Crop Protection* 22 (4), 579–588.
- Sharma, A., Johri, B.N., Sharma, A.K., Glick, B.R., 2003. Plant growth-promoting bacterium *Pseudomonas sp.* strain GRP 3 influences iron acquisition in mung bean (*Vigna radiata* L. Wilzeck). *Soil Biology and Biochemistry* 35 (7), 887–894.
- Tscharntke, T., Clough, Y., Wanger, T.C., Jackson, L., Motzke, I., Perfecto, I., et al., 2012. Global food security, biodiversity conservation and the future of agricultural intensification. *Biological conservation* 151 (1), 53–59.

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Recent Development of Patent in Indian Scenario With Special Reference to Microbial Patents

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8.1 INTRODUCTION

Patents are the legal rights granted for new inventions employing scientific and technical knowledge. The purpose of granting patents to the inventor or researchers by the government is to encourage inventive activities and also promote more novel works and ideas for the society or human beings. It provides a legal right to prevent other forms of practicing, that is, making, using, or selling the inventions covered by the patent. Patent provides protection to new invention from commercial competition for a limited period, known as the term of a patent (Besen and Raskind, 1991). There are a certain conditions and procedures have been followed to grant patent for an invention and it should be fulfilled:

- The invention must be novel, that is, the invention should not be recognized anywhere in the world on the date of filling of the application for patent.
- The inventive step, that is, the invention is not obvious to a person skilled in the field of the invention.
- The invention is useful to society.

If all of these criteria are met for an invention, then only any invention can be granted a patent.

The word patent originates from the Latin word *patere* which means “to lay open” to make available for public inspection (Koch, 2006). It is

shortened version of the term letters patent which was an open documents or instruments issued by a monarch or government granting exclusive right to a person, predating a modern patent system. Some available evidences suggest that some form of patent rights was recognized in ancient Greece. The first statutory patent system is considered to be the Venetian patent statute of 1450. The patent law in Indian perspective started with the inception of the Indian patent and design Act in 1911. Presently, it is governed by Patenting the Indian patents Act (1970) which came into force in the year 1972. The patent Act (1970) was again amended in 2005 where in product patent was extended to all field of technology including microorganism, microbial organism, chemicals, food, and drugs (Hamied, 1988; Vagadia, 2007).



8.2 WHAT CAN BE PATENTED?

For an invention to be patentable in India, the invention must be a new product or process, involving an inventive step and capable of being made or used in the industry. Further, the invention must also meet certain criteria in terms of novelty, inventive steps, and industrial and industrial applicability to be patented in India (Saha and Bhattacharya, 2011). In general, this means you must satisfy the following four requirements to qualify for a patent:

- Subject matter must be patentable.
- The invention must be novel.
- The invention must have some utility or usefulness.
- The invention must not be obvious.

8.2.1 Patentable subject matter

A patent cannot protect an idea. Instead the idea must be embodied in one or more of the following (<https://www.legalzoom.com/country/in>):

- A process or method (such as new way to manufacture concrete)
- A machine (something with moving parts or circuitry)
- A manufactured article
- A new composition
- An asexually reproduced and new variety of plant



8.3 TYPES OF PATENTS

There are three types of patents.

- **Utility patents:** This kind of patent may be granted for any new invention or discovery of any new and useful process, machine, article of manufacture or composition of matter, or any new and useful improvement thereof.
- **Design patents:** This patent may be granted for invention of a new, original, and ornamental design for an article of manufacture.
- **Plant patents:** This patent may be granted for invention or discovery of any distinct and new variety of plant.

8.3.1 Types of patent application filing in India

There are following types of patent application that can be filled in India for granting patent (<http://www.saroassociates.in/>);

- Ordinary patent application
- Convention patent application
- Patent corporation treaty (PCT) international patent application
- PCT National phase patent filing
- Application for patent of application
- Divisional patent application

8.3.2 Patent prosecution in India (<http://www.ipindia.nic.in/>)

- Patent filing
- Patent publication
- Patent examination
- First examination report
- Responding to objections
- Grant of patent



8.4 MICROBIAL PATENTS IN INDIAN SCENARIO

Patent Act of India 1970, section 2(1) defined “invention” as a new and useful manner of manufacture or a substance produced by manufacture (Adelman and Baldia, 1996). The patent office accepted the

patentable subject matter only if it is resulting from physical or nonliving substance. According to section 3(j) of the Act, the plants and animals in whole or in part thereof were left out from the patent. As India joined the Budapest Treaty on December 17, 2001, thereafter the position of microbial patents had become clear after the amendment of Indian patents Act 1970 in 2002 (Sekar and Kandavel, 2002; Hamied, 1988). In recent past, it has been found a tremendous increase in the application number for the patent related to living matter. This is due to the fact that in last few decades, there has been witnessed a phenomenal growth in the researches of biotechnology and microbiology (Gavrilescu and Chisti, 2005). Inventions which involved some kind of living matter were already protected by a patent law. Microorganism plays an important role in our society and advancement in the field of life science, microorganism, and microbial processes have made it possible to economically exploit their potential which has in fact triggered a lot of debate about patenting of microorganism (<http://iprlawindia.org/>).



8.5 STATUS OF MICROBIAL PATENTING

Patent laws were initially framed from chemical, mechanical, and electrical invention point of view but later on information technology, electronics, pharmacy, and biotech emerged as chief field of intellectual property right (IPR) resulting in change in prior law. Although first patent related to microorganism granted in 1873 but after that there was no any significant development had been found in microorganism patenting, but after the emergence and advancement of genetic engineering, there have been found an overflow of patent claims associated with microbes.

Some of the major debatable concerns besides the questions what is meant by term microorganism are

- Can a microorganism be patented in itself? If yes should it be in accessible in live form.
- Are new applications of preexisting microbes patentable?
- Can isolated microorganism that exists in nature patented?
- Are microorganism products patentable?
- Are processes of producing microorganism patentable? If yes what is process?

Patentability of microorganism is governed by various international and national legislation and interpretation in case of laws. After Trade-related aspect of intellectual property rights (TRIPS) agreement, it is well settled that microorganisms are patentable and member countries are under obligation to provide patent of microorganism (Correa, 2007). This agreement made obligatory to provide patent for microorganism per se as well as for invention based on microbiological process. The Indian laws are yet to be amended to make provisions in line with these agreements for patenting of microorganism. In the patent law of most countries, the microorganism has not been clearly defined. As a result, there is a flexibility in determining what can be patented in microorganism. For example, a particular bacterial strain may first be isolated in a natural sample and thereafter reproduced by laboratory cultivation (Nair 1999). In case of microorganism is known species or a variant, it may be necessary to indicate literature that discloses the said known species and to describe its scientific name and the reason why the particular microorganism is identified as the known species or variant. A number of patent are now granted for microbiological invention. The invention covered a method and composition for genetically transferring microorganisms, particularly bacteria to provide them with a wide-ranging capability of generating nucleic acid and proteins like medically important or commercially useful enzyme. These bacteria may also find application in production of drugs, hormone, and antibiotics such as nitrogen fixation, fermentation, and utilization of specific feed stocks (Janke, 2003).

Patenting of microbes potentially raise several ethical and moral issues; such as the developments in patenting could be tantamount to owning living creature. The utility of invention should be for humanity; any microbe is a property of nation, is it appropriate to properties a particular microbe to particular person and devoid others from its benefits. Microbes are common heritage and it is not moral perspective to devoid others. Time limit of 20-month years given to patentee is too much. Besides, it is negative rights in logic that it means injunction (Webber, 2006).



8.6 REQUIREMENTS FOR MICROBIOLOGICAL PATENT APPLICATION

A patent is monopoly granted to an inventor by the state in exchange for invention disclose. For this reason, patent laws require that

the description of the patent application discloses the invention in a manner sufficiently clear and complete for the invention to be carried out by a person skilled in the art. Apart from the usual requirement for, this kind of invention has some specific requirements patentability (Seriñá and Toledo, 1999). First, the description shall contain all the information the application has about the characteristics of the deposited biological materials. Also, the biological materials have to be deposited no longer than the date on which the patent application filed with a depositary institution recognized under the Budapest Treaty on the International recognition of the deposit of microorganism for the purpose of patent procedure (Jauhar and Narnaulia, 2010).

8.6.1 Types of patentable microbiological invention

Most patent legislation distinguishes three kinds of inventions depending on the type of subject to be protected.

1. Invention that protects a product.
2. Invention based on the process to obtain a product.
3. Invention referring to the use of a product. It is also possible to include in the same invention claims of the three types; for example,
 - a. A claim for a product.
 - b. A claim for a process to obtain that product.
 - c. A claim aimed to the use of product.

In the field of microbiology, both European and Spanish legislation admit the patentability of product obtained by microbiological process. Following this reasoning, microorganism per se is also patentable subject matter. The guideline for examination in the European patent office specifies that “propagation of the microorganism itself is to be construed as a microbiological process; consequently, the microorganism can be protected per se as it is a product obtained by a microbiological process” (Seriñá and Toledo, 1999; Jauhar and Narnaulia, 2010).



8.7 CRITICAL ISSUES ON MICROBIAL PATENTS

1. Microorganism needs to be defined and because of its distinct nature it should be treated individually.
2. Different groups of microbes should be defined clearly.

3. Patenting of a microbial consortia, should be favored rather than patenting single microorganism.
4. Develop a plan to get maximum benefits from patenting microorganism isolated from India.
5. Efforts for documentation and patenting the traditional knowledge on use of microbes.



8.8 CONCLUSION AND FUTURE PROSPECTIVE

Currently most of the researchers and readers are not aware about patent and IPR, because of this some novel and high value results of long research have been lost. Therefore, there is an urgent need to make the researchers acquainted with the role and mode of patentability of microbiological inventions to acknowledge their hard work. Now a days, the immediate publishing of research in scientific journals and magazines destroyed the novelty of a possible patent and they do not apply for a patent rather they contain novel inventions and already had a chance for patentability requirements (Seriñá and Toledo 1999). Patenting in life science or microbiology may have multifaceted that relate to the use of intellectual property rights concept to get the rights of ownership, dissemination and transfer, etc. Internationally the TRIPS provide patent protection to the microbiological, biological, and nonbiological production of plants and animals (Nair and Ramachandranna, 2010).

This may be drawback of TRIPS because it does not provide a definite definition of microorganism as per concerned about the patent protection of microorganism, since as per TRIPS microorganisms are present in nature and their discovery can not be called as invention whereas genetically modified microorganism comes under the category of invention because of human input. Genetically modified microorganism may perform any number of activities, hence patenting of this genetically modified microorganism will result in blocking of further research on that microorganism.

Currently there is huge interest of biotech industry for the novel innovation and endeavor in the field of life science, microbiology, and pharmaceutical industries to increased inventions for the welfare of human beings. But without an efficacious protection system of patents

and IPRs, the vast reservoir of such information may remain a secret. So, there is the need for a systematic, easy, and substantial patent system for the protection of the research concerning microorganisms.

REFERENCES

- Adelman, M.J., Baldia, S., 1996. Prospects and limits of the patent provision in the TRIPs Agreement: the case of India. *Vand J Transnat'l L* 29, 507.
- Besen, S.M., Raskind, L.J., 1991. An introduction to the law and economics of intellectual property. *J Econ Perspect* 5 (1), 3–27.
- Correa, C., 2007. Trade related aspects of intellectual property rights; a commentary on the TRIPs agreement. OUP Catalogue.
- Gavrilescu, M., Chisti, Y., 2005. Biotechnology—a sustainable alternative for chemical industry. *Biotechnol Adv* 23 (7–8), 471–499.
- Hamied, Y.K., (1988), November. The Indian Patents Act, 1970 and the Pharmaceutical Industry. In Cipla Ltd, Bombay, National Seminar on Patent Laws, New-Delhi (Vol. 22).
- <<http://iprlawindia.org/>>
- <<http://www.ipindia.nic.in/>>
- <<http://www.saroassociates.in/>>
- <<https://www.legalzoom.com/country/in>>
- Janke, T., 2003. Case Studies on Intellectual Property and Traditional Cultural Expressions. WIPO, Geneva.
- Jauhar, Ameen, Narnaulia, Swati, 2010. Patenting Life the American, European and Indian Way. *J. Intellect. Property rights* 15, 55–65.
- Koch, L., 2006. Patently important. *Chem Aust* 73 (6), 4.
- Nair, A.S., 1999. Intellectual property rights (IPR): Indian scenario. *Everyman Sci* 34 (2), 58–61.
- Nair, R.B., Ramachandranna, P.C., 2010. Patenting of microorganisms: systems and concerns. *J Commer Biotechnol* 16 (4), 337–347.
- Saha, C.N., Bhattacharya, S., 2011. Intellectual property rights: an overview and implications in pharmaceutical industry. *J Adv Pharm Technol Res* 2 (2), 88.
- Sekar, S., Kandavel, D., 2002. Patenting microorganisms: towards creating a policy framework. *J. Intellect. Prop. Rights* 7, 211–221.
- Seriñá, I., Toledo, C., 1999. Microbiological patents. *Int Microbiol* 2 (3), 199–200.
- Vagadia, B., 2007. Intellectual property rights (IPR) Outsourcing to India—A Legal Handbook. Springer, Berlin, Heidelberg, New York, pp. 137–145.
- Webber, P.M., 2006. Patenting of microorganisms. *Nat Rev Drug Discov* 5, 13.

FURTHER READING

- Maselkar, R.A., 2001. Intellectual property right and the third world. *Curr Sci* 81, 955–965.



Evidence for Widespread Microbivory of Endophytic Bacteria in Roots of Vascular Plants Through Oxidative Degradation in Root Cell Periplasmic Spaces

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9.1 INTRODUCTION

There is growing evidence that all plants are inhabited by a plethora of microbes (Stone et al., 2000; Arnold and Lutzoni, 2007; Rosenblueth and Martínez-Romero, 2006; Magnani et al., 2010; Compant et al., 2010; Johnston-Monje and Raizada, 2011). These components of the plant microbiome are both bacterial and fungal and may exist on plant surfaces and interiors. How microbes interact with one another, and with host plants, is currently not well understood, although we do have fragmentary knowledge. For example, research on individual components of the microbiome indicates that the microbiome inhabitants may enhance a host plant's resistance to biotic and abiotic stresses (Kloepper, 1993; Redman et al., 2002; Clay et al., 2005; Waller et al., 2005; Clarke et al., 2006; Weber et al., 2007; Kuldau and Bacon, 2008; Rodriguez et al., 2009; Álvarez-Loayza et al., 2011; Bacon and Hinton, 2011; Hamilton et al., 2012; Torres et al., 2012; Doty, 2017). These studies clearly

indicate that the microbiome may possess some defensive properties that benefit plant hosts. There are also indications that plant microbiomes possess nutritional properties (Hurek et al., 1994; Döbereiner, 1992; Döbereiner et al., 1994; James et al., 1994; Puente and Bashan, 1994; Glick, 1995; James and Olivares, 1998; James, 2000; Reinhold-Hurek and Hurek, 2011). One such example is the phenomenon of “associative nitrogen fixation” where endophytic diazotrophic bacteria in the microbiome fix nitrogen and stimulate plant growth. Some scientists posit that this phenomenon is responsible for efficient plant growth in crops such as sugarcane, rice, wheat, and corn (James, 2000; Urguiaga et al., 1992; Taulé et al., 2012). However, most research on plant microbiome nutritional effects on plant growth and development to date has involved experiments conducted on individual bacteria that are applied to plants. Consequently, we have limited information regarding other microbiome inhabitants.

Precisely how host plants obtain nutrients from the microbes that colonize them has long been an unanswered question (James, 2000). Paungfoo-Lonhienne et al. (2010a,b) provided the first evidence of a mechanism for the transfer of nutrients from microbiome microbes to plants by demonstrating that tomato plants and *Arabidopsis thaliana* are capable of microbivory through endocytosis and degradation of microbes within root cells. Microbivory is generally known to occur among heterotrophic protozoans and simple animals where the eukaryotic consumer engulfs and degrades bacteria as a nutrient source (Mikola, 1998). More recently, White et al. (2012) demonstrated the lysis of diazotrophic bacteria on surfaces of grass root hairs and root epidermal tissues. The process of lysis involved, at least in part, secretion of reactive oxygen onto bacteria to degrade/oxidize bacteria, and for that reason the process was termed “oxidative nitrogen scavenging.” Collectively these studies suggest that at least some plants have the capability to acquire nutrients through the lysis of microbes in the microbiome. Moreover, widespread microbivory in plants for nutrient acquisition from microbes could have far reaching consequences. To be specific, understanding this mechanism could lead to the development of new strategies for plant cultivation that use microbes as nutritional supplements instead of inorganic fertilizers (Kraiser et al., 2011; Paungfoo-Lonhienne et al., 2012).



9.2 SEEDLING SURVEY, SEED TRANSMISSION, AND BACTERIAL DISTRIBUTION IN SEEDLING TISSUES

Bacteria are seed transmitted, although it is difficult to determine precisely where bacteria are vectored in seeds. There are two options: 1. Bacteria may be embedded on/in the seed surface layers, perhaps in resistant bio-films; or 2. Bacteria may occur within seeds, perhaps in the embryo itself (Frank et al., 2017; Rodríguez et al., 2017). In the grasses rigorous disinfection appeared to drastically reduce bacteria in seedling root tissues (White et al., 2015; White et al., unpublished). This suggests that most of the bacteria in these plants are vectored on the surface of seeds (White et al., 2012). It is possible that seedlings of all plant species may also recruit bacteria from the environment. Seedlings growing in soils may acquire diverse recruited environmental bacteria (Frank et al., 2017; Rodríguez et al., 2017). Future studies will be needed to resolve questions of the ecologies of the seedling bacteria.

We conducted a survey of seedlings of 36 species of plants by collecting seeds from numerous sources, washing them with continuous agitation in three changes of sterile water (5 minutes each change) to remove soil and debris, germinating them on agarose and staining them in 2.5 mM diaminobenzidine tetrachloride (DAB; Sigma-Aldrich, USA) by flooding plates with the stain for 10–12 h. DAB-stained roots were counter stained with aniline blue and observed under the light microscope (White et al., 2014; White et al., 2017). DAB enables visualization of reactive oxygen produced around intracellular bacteria (White et al., 2014), showing both presence of bacteria and action of reactive oxygen on them. Aniline blue stains proteins in bacterial cytoplasm and shows blue-stained bacterial contents in bacteria that are not fully oxidized; swollen bacteria without internal blue staining indicates fully oxidized bacteria. For *Polypodium polypodioides* young plants were collected from natural populations, stained, and examined microscopically.

In our survey of seedlings, bacteria were mostly found in root tissues—but were also sometimes observed in shoot tissues (Table 9.1). In roots, the bacteria were present frequently in parenchyma and root hair cells located in the periplasmic space beneath the cell walls where they were seen to lyse [Fig. 9.1(C–F), Fig. 9.2(A–D), Fig. 9.3(B), Fig. 9.4(D,E), Fig. 9.5(B–E)]. Thomas and Reddy (2013) observed

Table 9.1 Survey of plant species for evidence of oxidation/degradation of bacteria in seedling root cells

Species	Family	Origin	Bacteria isolated	Cells showing bacterial degradation
<i>Agave chrysantha</i>	Agavaceae	Sonoran desert, AZ	Unidentified	Root hairs, Root epidermis, Root cap
<i>Agave palmeri</i>	Agavaceae	Sonoran desert, AZ	<i>Klebsiella</i> sp. (White et al., 2014)	Root hairs, Root epidermis, Root cap
<i>Agave schottii</i>	Agavaceae	Sonoran desert, AZ	Unidentified	Root hairs, Root epidermis, Root cap
<i>Amaranthus viridis</i>	Amaranthaceae	Commercial source, USA	Unidentified	Root hairs, Root epidermis
<i>Apium graveolens</i>	Apiaceae	Commercial source, USA	Unidentified	Root hairs, Root epidermis
<i>Brassica napus</i>	Brassicaceae	Commercial source, USA	Unidentified	Root hairs, Root epidermis
<i>Celastrus orbiculatus</i>	Celastraceae	East Brunswick, New Jersey	Unidentified	Root hairs, Root epidermis, Root cap
<i>Coriandrum sativum</i>	Apiaceae	Commercial source, Mexico	Unidentified	Root hairs, Root epidermis, Root cap
<i>Cucurbita pepo</i>	Cucurbitaceae	Commercial source, USA	Unidentified	Root hairs, Root epidermis, Root cap
<i>Citrullus colocynthis</i>	Cucurbitaceae	Commercial source, Nigeria	Unidentified	Root hairs, Root epidermis, Root cap
<i>Cereus repandus</i>	Cactaceae	Bonaire, Dutch Antilles	<i>Achromobacter xylosoxidans</i> (White et al., 2014)	Root hairs, Root epidermis, Root cap
<i>Cynodon dactylon</i>	Poaceae	Commercial source, USA	Unidentified	Root hairs, Root epidermis, Root cap
<i>Dahlia</i> sp.	Asteraceae	Commercial source, USA	<i>Bacillus</i> sp. (Li et al., 2015)	Root hairs, Root epidermis
<i>Fallopia japonica</i>	Polygonaceae	South River, New Jersey, USA	<i>Bosea thiooxidans</i> (White et al., 2017)	Root hairs, Root epidermis

(Continued)

Table 9.1 (Continued)
Species

	Family	Origin	Bacteria isolated	Cells showing bacterial degradation
<i>Fimbristylis cymosa</i>	Cyperaceae	Bonaire, Dutch Antilles	Unknown	Root hairs, Root epidermis, Root cap
<i>Festuca arundinacea</i>	Poaceae	Commercial, USA	<i>Pantoea agglomerans</i> White et al. (2012)	Root hairs, Root epidermis
<i>Hedera helix</i>	Araliaceae	New Jersey, USA	<i>Bacillus amyloliquefaciens</i> (Soares et al., 2015)	Root hairs, Root epidermis
<i>Leersia oryzoides</i>	Poaceae	New Jersey, USA	<i>Pantoea</i> spp., <i>Pseudomonas</i> sp. (Verma et al., 2017b)	Root hairs, Root epidermis, Root cap
<i>Lolium perenne</i>	Poaceae	Commercial source, USA	<i>Bacillus</i> sp.	Root hairs, Root epidermis
<i>Lonicera japonica</i>	Caprifoliaceae	New Jersey, USA	Unknown	Root hairs
<i>Lycopersicum esculentum</i>	Solanaceae	Commercial source, USA	<i>Acinetobacter</i> sp., <i>Micrococcus</i> <i>luteus</i> White, (Unpublished)	Root hairs, Root epidermis, Root cap
<i>Moringa oleifera</i>	Moringaceae	Commercial source, USA	<i>Citrobacter</i> sp., <i>Bacillus</i> sp., <i>Klebsiella</i> sp. (White, Unpublished)	Root hairs, Root epidermis, Root cap
<i>Oryza sativa</i>	Poaceae	Commercial source, USA	Verma et al. (2017a)	Root hairs, Root epidermis, Root cap
<i>Panicum virgatum</i>	Poaceae	East Brunswick, New Jersey, USA	<i>Burkholderia</i> sp. (White et al., 2014)	Root hairs, Root epidermis
<i>Poa annua</i>	Poaceae	Penn State Cultivar Selection, From David Huff	Unidentified	Root hairs, Root epidermis, Root cap
<i>Phaseolus acutifolius</i>	Fabaceae	Commercial source, USA	Unidentified	Root hairs, Root epidermis, Root cap
<i>Phragmites australis</i>	Poaceae	New Jersey, USA	<i>Pseudomonas</i> spp., <i>Pantoea</i> sp. White et al. (2017)	Root hairs, Root epidermis, Root cap

(Continued)

Table 9.1 (Continued)
Species

	Family	Origin	Bacteria isolated	Cells showing bacterial degradation
<i>Polypodium polypodioides</i>	Polypodiaceae	Panama	Unidentified	Root hairs
<i>Rhus radicans</i>	Anacardiaceae	Middlesex Co., New Jersey, USA	<i>Sphingomonas</i> sp. (White et al., 2014)	Root hairs, Root epidermis, Root cap
<i>Ritterocereus griseus</i>	Cactaceae	Bonaire, Dutch Antilles	Unidentified	Root hairs, Root epidermis
<i>Rumex crispus</i>	Polygonaceae	New Jersey, USA	Unidentified	Root hairs, Root epidermis, Root cap
<i>Taraxacum officinale</i>	Asteraceae	South River, New Jersey, USA	Unidentified	Root hairs, Root epidermis, Root cap
<i>Triticum aestivum</i>	Poaceae	Commercial source, USA	Unidentified	Root hairs, Root epidermis, Root cap
<i>Typha latifolia</i>	Typhaceae	New Brunswick, New Jersey, USA	Unidentified	Root hairs, Root epidermis, Root cap
<i>Vaccinium oxycoccos</i>	Ericaceae	New Jersey	Unidentified	Root hairs, Root epidermis, Root cap
<i>Yucca schottii</i>	Agavaceae	Sonoran desert, USA	<i>Klebsiella</i> sp. White et al. (2014)	Root hairs, Root epidermis, Root cap

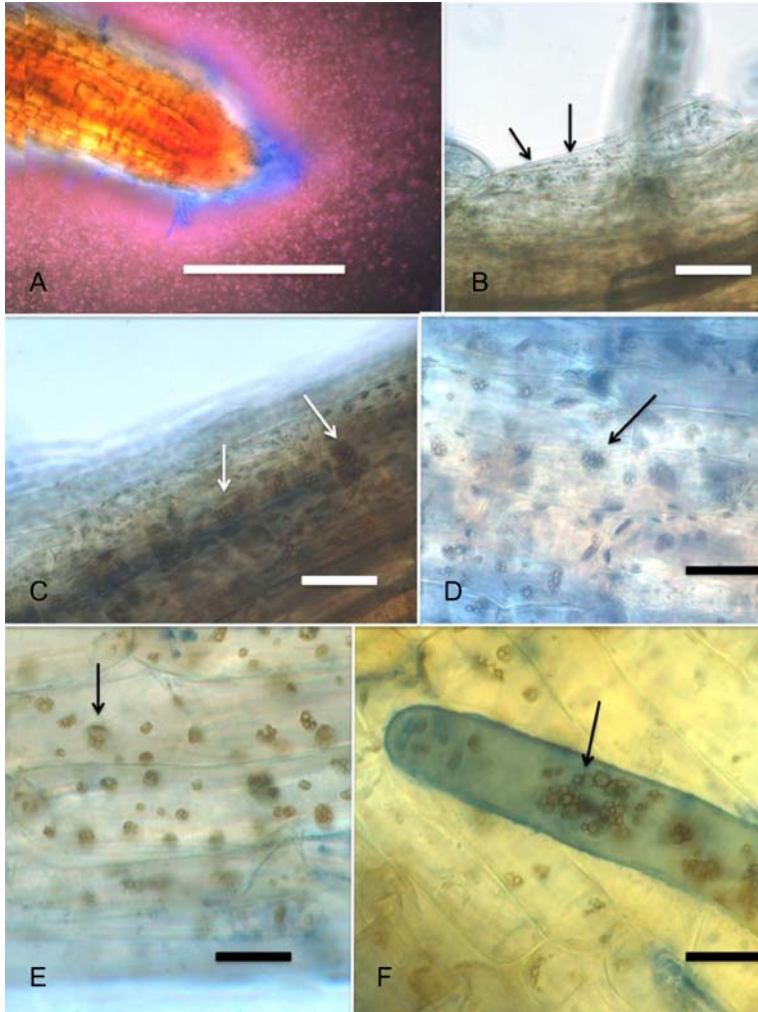


Figure 9.1 (A–F) Cattail (*Typha angustifolia*) seedling germinated on agarose. (A) Root tip showing pink cloud of bacteria surrounding the meristem in the zone of intracellular colonization (bar = 1 mm). (B) Epidermal cell near the root tip meristem showing blue-stained bacteria (arrows) in the periplasmic space of the epidermal cell. Intracellular bacteria are evident as small blue specks on the plasma membrane of the cell (bar = 10 μ m). (C–E) Root axis showing epidermal parenchyma cells containing brown clusters of oxidizing bacteria (arrows) in the periplasmic space of cells. Root was stained with DAB to visualize reactive oxygen around oxidizing bacteria (bar = 10 μ m). (F) Root hair showing internal cluster of oxidizing bacteria (arrow) in the periplasmic space; the tissue was stained with DAB to show H_2O_2 (brown).

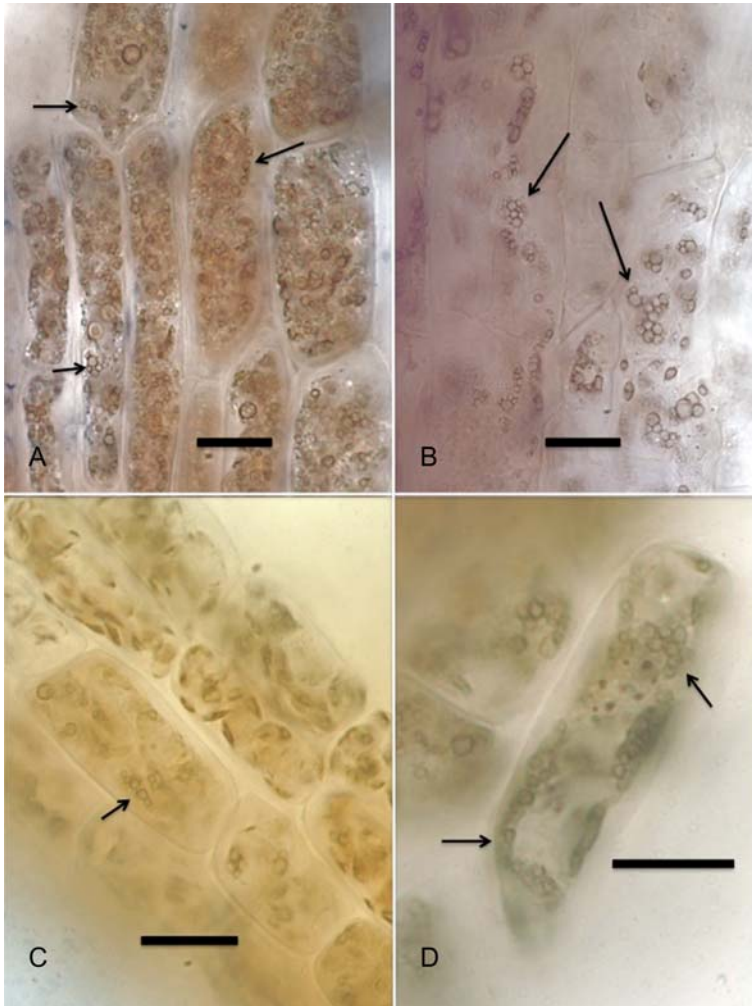


Figure 9.2 (A–D) Roots belonging to species of family Cucurbitaceae showing internal oxidizing bacteria. (A and B) Root cortex cells showing oxidizing bacteria (arrows) in the periplasmic spaces of roots of egusi melon (*Citrullus colocynthis*) stained with DAB (bar = 10 μm). (C) Root cortex cells showing oxidizing bacteria (arrows) in the periplasmic spaces of roots of acorn squash (*Cucurbita pepo*) stained with DAB. (D) Sloughed-off root cap cell of acorn squash seedling stained with DAB showing oxidizing bacteria (arrow) in the periplasmic space (bar = 10 μm).

colonization of periplasmic spaces of several lines of bananas (See also [Thomas and Soly, 2009](#)). In root tissues, nonlysed bacteria were often located closer to the root tip meristem [Fig. 9.1(A,B)] with swelling and lysis of bacteria more pronounced in cells as they differentiated

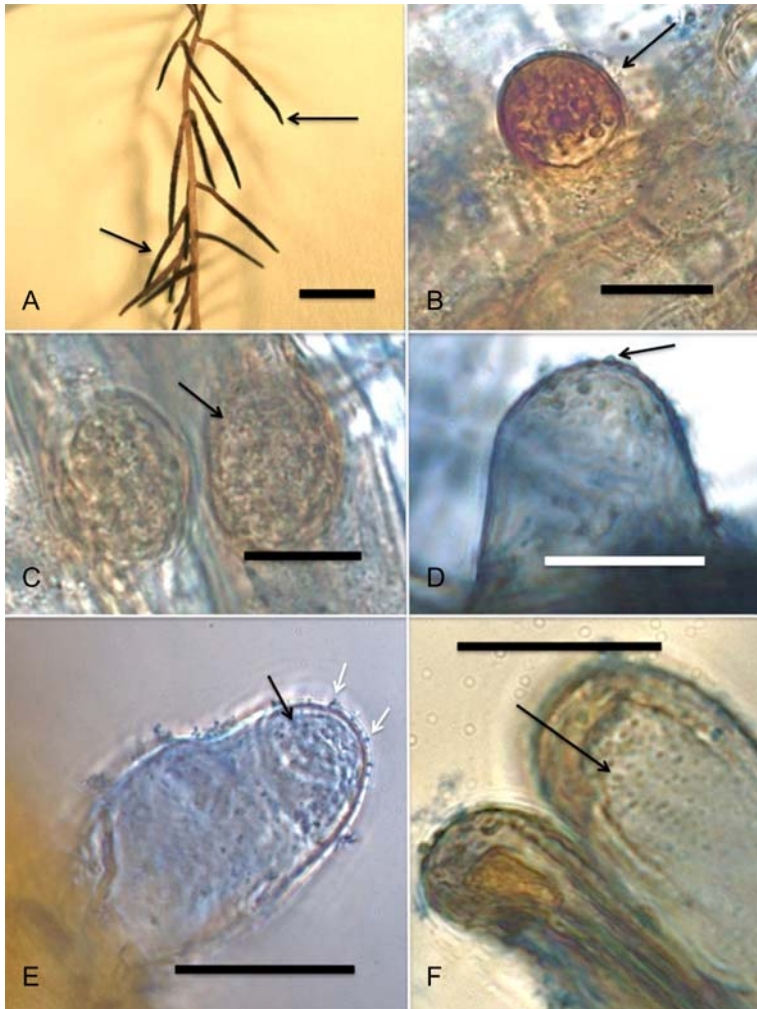


Figure 9.3 (A–F) English Ivy (*Hedera helix*) showing root hairs with intracellular bacteria. (A) Root of English ivy propagated in the laboratory from cuttings then stained with DAB to show high reactive oxygen activity (brown coloration) in the lateral roots (arrows) that bear numerous root hairs (bar = 1 cm). (B) Root hair initial (arrow) showing high reactive oxygen activity (brown coloration) and internal oxidizing spherical bacteria (bar = 10 μ m). (C) Root hair initials (arrow) showing numerous internal bacteria (bar = 10 μ m). (D) Root hair initial showing bacterial L-forms (arrow) exiting hair at the tip (bar = 10 μ m). (E) Root hair initial showing numerous internal bacteria (black arrow) clustered in the periplasmic space at the hair tip (bar = 10 μ m). Exit pores (white arrows) are visible in wall at the hair tip. (F) Root hair showing bacteria (arrow) beneath the wall at the hair tip (bar = 10 μ m).

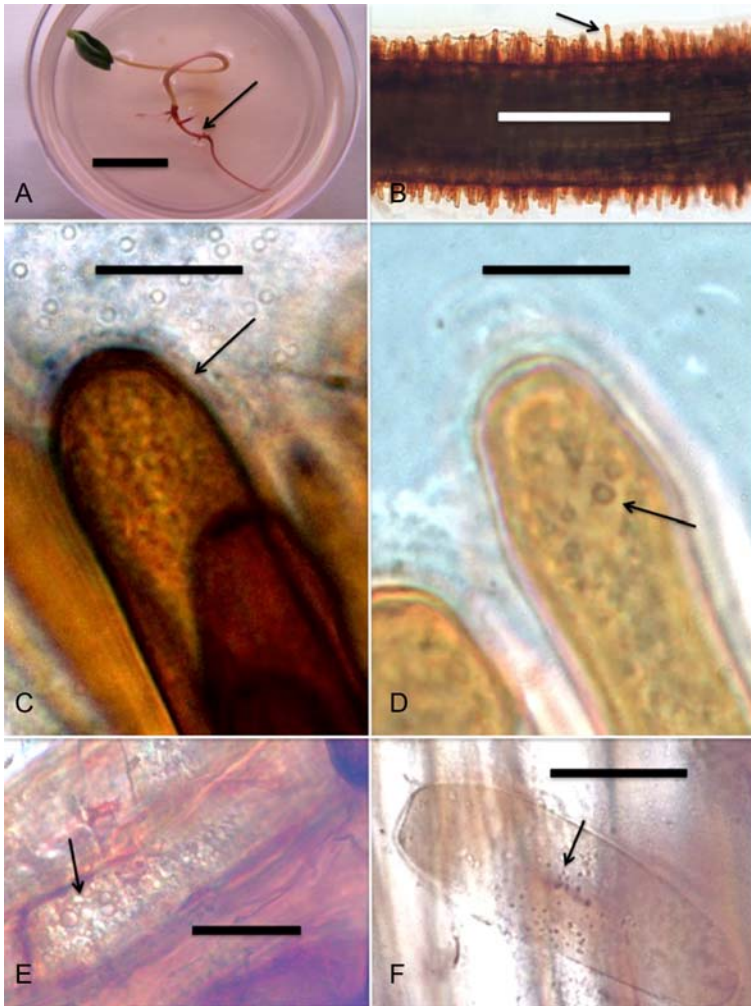


Figure 9.4 (A–F) Poison Ivy (*Rhus radicans*). (A) Seedling of *Rhus radicans* growing on agarose showing characteristic red roots (arrow) (bar = 1 cm). (B) Roots growing in agarose showing numerous root hairs (arrow) (bar = 1 mm). (C) Root hair (arrow) showing granular appearance internally due to internal bacteria (bar = 10 μm). (D). Root hair stained with DAB showing bacteria (arrow) in a vesicle surrounded by red to brown ring of reactive oxygen (bar = 10 μm). (E) Root axis parenchyma cell showing oxidizing bacterial L-forms (arrow) in the periplasmic space (bar = 10 μm). (F) Sloughed-off root cap cell showing bacteria (arrows) internally (bar = 10 μm).

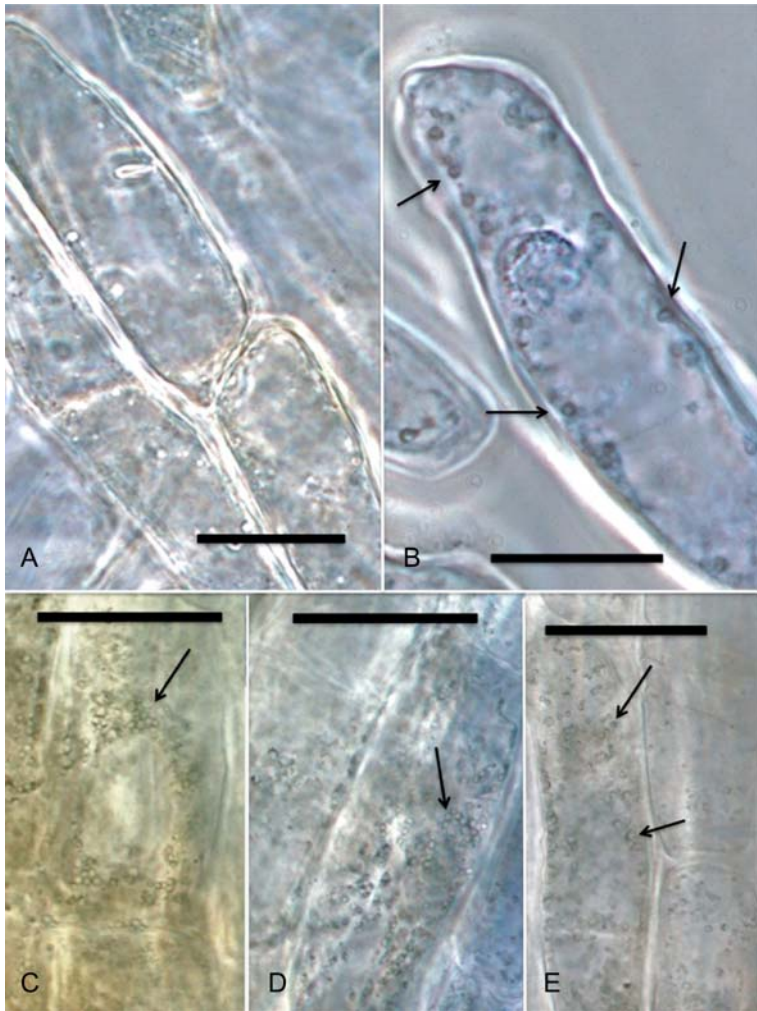


Figure 9.5 (A–E) Corn (*Zea mays*) root cells stained with DAB and aniline blue to show internal bacteria. (A) Sloughed-off root cap cells of modern white corn seedling showing absence of internal bacteria in the periplasmic space of cells (bar = 10 μm). (B) Root cap cells from tropical corn seedling showing bacteria (arrows) in vesicles with a ring of reactive oxygen (reddish ring) surrounding bacterial cells (bar = 10 μm). (C–E) Root epidermal parenchyma cells from tropical corn seedling stained with DAB showing oxidizing bacteria (arrows) in cells (bar = 15 μm).

[Fig. 9.1(C–F)]. This pattern of distribution of bacteria may be an indication that bacteria proliferate around the root tip meristem of seedlings [see Fig. 9.1(A)] and colonize the meristematic cells [Fig. 9.1(B)] where cell walls are relatively undeveloped. Bacteria that proliferate in root and shoot-tip meristems are distributed in all parts of the plant; when plants produce fruits they may be colonized by the endosymbiotic bacteria.



9.3 EVIDENCE FOR MICROBIVORY IN DIVERSE VASCULAR PLANT FAMILIES

In our survey, we found evidence of intracellular bacteria in all species examined [Table 9.1; Fig. 9.1(B–F), Fig. 9.2(A–D), Fig. 9.3(B), Fig. 9.4(D, E), Fig. 9.5(B–E)], including some 36 species of plants distributed in 20 families of vascular plants. Ferns and seed plants were found to internalize and oxidize bacteria in root cells. In all species, bacteria appeared to internalize in root cells at root tip meristem as walled cells, only to become wall-less L-forms once inside the periplasmic space between plant cell wall and plasma membrane [Fig. 9.2(A–D)]. Even aerial roots of vines, including English Ivy (*Hedera helix*) and Poison Ivy (*Rhus radicans*), were seen to internalize and oxidatively-degrade bacteria within root cells [Fig. 9.3(A–F), Fig. 9.4(A–F)]. An exception was white corn (*Zea mays*), a modern corn hybrid, where examination of seedlings did not reveal abundant bacterial internalization in root cells [Fig. 9.4(F)]. However, tropical corn (*Zea mays*) and Indian corn (*Zea mays*), both less intensively selected than modern hybrids, were found to contain abundant bacteria in seedling root cells [Fig. 9.5(B–E)]. It could be that the high dependence of modern hybrid corn varieties on inorganic fertilizers may be the result of the loss of symbiotic bacteria that function carry nutrients into plants.



9.4 NUCLEAR COLONIZATION

Bacteria resisted degradation longer when they entered nuclei of plant cells. Lysed bacteria were frequently found in cell cytoplasm just outside the nuclear envelope [Fig. 9.5(C)] and intact bacteria within the nuclei. It is

possible that bacteria survive longer within nuclei because the nucleus is an area of the cell where reactive oxygen levels are low and lysis generally does not occur. Possibly, bacteria may produce nucleomodulins to control gene expression of the cell to reduce oxidative processes or alter activities of the cell in a way that favors the endosymbiotic bacteria (Bierne and Cossart, 2012). For example, nucleomodulins are produced by *Agrobacterium tumefaciens*, another species of Proteobacteria. Nuclear colonization also occurs in protozoans where alpha-Proteobacteria of the family Holosporaceae are phagocytized by the host protozoan and transported to the nucleus where bacteria multiply. Nuclear colonization by bacteria was also described in the symbiotic system involving endonuclear beta-Proteobacteria and the dinoflagellate *Gyrodinium instriatum* (Alverca et al., 2002). In this system, dividing bacteria were observed in the nucleus. Intra-nuclear bacteria were released to the cytoplasm where they were often degraded (Alverca et al., 2002). These authors also proposed that nuclear colonization was a strategy to escape digestion that occurred in the cytoplasm of the alga. It is possible that bacteria in root cells may enter nuclei as a way to escape reactive oxygen degradation that occurs on host cell membranes in the cytoplasm.



9.5 BACTERIAL MOVEMENT IN PLANT CELLS

Research on the Holosporaceae suggests that the bacteria are able to move through the host cell's cytoplasm to the nucleus through use of actin filaments (Sabaneyeva et al., 2009). However, this mechanism may not be universal for intracellular bacteria. In making observations on unstained living seedling roots of the sedge *Fimbristylis cymosa* and the grass *Festuca arundinacea* movement of bacteria was observed within root hairs. Bacteria located in the periplasmic space appeared to flow along the length of root hairs. The rate of flow was 8–11 $\mu\text{m}/\text{second}$ for the sedge *F. cymosa*. This movement may have been due to cyclosis within the root hairs. Paungfoo-Lonhienne et al. (2010a,b) also reported movement of intracellular microbes in root hairs of *A. thaliana*. Bacterial movement was not observed in stained seedlings since stains DAB and aniline blue with lactophenol generally stopped cyclosis in cells. It is likely that movement within cells is the norm for the intracellular bacteria. This intracellular movement may permit the bacteria to spread to all parts of the host cell. Constant movement of intracellular bacteria may also reduce the effects of reactive oxygen and lysing enzymes on bacteria.



9.6 BACTERIAL COLONIZATION OF SEEDLING ROOTS OF *PANICUM VIRGATUM*

Experiments were conducted to evaluate colonization of seedling roots using a strain of *Burkholderia gladioli* isolated from germinating seedlings of *Panicum virgatum* (White et al., 2014). Through surface disinfection seeds free of, or with reduced levels, of bacteria were obtained with which we conducted seedling infection experiments. On inoculation of seedlings with bacterial suspensions colonization of seedling roots was observed. This was accompanied by a shape change in the structure of bacteria from rod- to sphere-shaped (White et al., 2014). Paungfoo-Lonhienne et al. (2010a,b) in a study of *Escherichia coli* entry into *Arabidopsis* seedlings found that entry into cells was accompanied by up-regulation of the plant cell wall-related enzymes, including expansins, cellulases, pectinases, xyloglucan endotransglycosidases, and cellulose synthases. Involvement of host enzymes in the entry of bacteria into cells suggests that plant cells are engaging in phagocytosis to acquire bacteria. However, it is also possible, at least in some cases, that the bacteria themselves produce cell wall loosening and degrading enzymes to colonize the interior of plant cells. Other symbiotic bacteria are thought to enter plant cells using their own cell wall degrading enzymes. Kovtunovych et al. (1999) demonstrated that the capacity of *Klebsiella oxytoca* to endophytically colonize wheat plants correlated with its ability to produce pectinases. Compant et al. (2005) demonstrated that *Burkholderia sp.* required use of cell wall degrading enzymes endogluconase and polygalacturonase to internally colonize grapes.



9.7 CHANGE IN BACTERIAL SHAPE

The change of cell shape from rod-like to spherical for *Burkholderia* cells colonizing seedling roots of *P. virgatum* is likely the result of interaction between the plant and bacterium (Beran et al., 2006). Shape transformations also occur in bacteria that intracellularly colonize animals (Beran et al., 2006). The spherical, often intracellular, forms are referred to as L-forms or cell wall deficient forms (Beran et al., 2006). L-forms of bacteria are found in healthy intestinal tracts of animals and have been implicated as causal agents of human diseases such as Crohn's disease, ulcerative

colitis, Sarcoidosis, pulmonary tuberculosis, Hodgkin's disease, and several other human diseases (Wall et al., 1993; Beran et al., 2006). Bacteria lose their cell walls due to loss of structural wall components, resulting in spherical bacterial cells. It has also been suggested that bacteria form L-forms in order to evade host defenses (Beran et al., 2006).

In plants, artificially generated L-forms of bacteria have been shown to enter seedling root tissues and establish intracellular symbiotic growth (Amijee et al., 1992; Daulagala and Allan, 2003). In several plants establishment of endosymbiotic L-forms of bacteria resulted in enhanced resistance to a range of plant pathogens, although the mechanism of enhanced resistance has not been clarified (Amijee et al., 1992). The majority of the intracellular bacteria we observed in seedlings were spherical forms (Table 9.1). When bacteria were isolated from seedlings they were invariably rod forms in culture. Reversion to the walled cell shape is common when L-forms are isolated onto nutrient media (Beran et al., 2006).



9.8 EVIDENCE FOR INCREASED NITROGEN ASSIMILATION BY BACTERIA IN PLANTA

Research on nitrogen fixation by diazotrophic bacteria applied to plant tissues has implicated roots rather than shoots as the location where nitrogen fixation (*nif*) genes are expressed in bacteria, suggesting that bacteria associated with roots are active in nitrogen fixation (Hurek et al., 1997; Rosenblueth and Martínez-Romero, 2006). Soares et al. (2016) conducted ^{15}N gas-tracking experiments using plants of *Phragmites australis* with and without addition of endophytic bacteria and similarly found that increased nitrogen assimilation into roots translated into increased plant growth, while increased assimilation into leaves was not accompanied by increased growth. Whether the naturally-occurring intracellular bacteria in seedling roots are active in nitrogen fixation is yet to be determined. It seems evident based on our observations and those of others that some of the nutrients acquired by vascular plants are acquired through lysis of bacteria that become intracellular in roots (Beltrán-García et al., 2014). Research by Paungfoo-Lonhienne et al. (2010a) is suggestive of a mechanism in plants whereby they are adapted to obtain nutrients from intracellular bacteria. These investigators found that in *A. thaliana* exposure of seedling roots to nucleic acids turns on a pathway

that enables plants to obtain nitrogen from proteins (Paungfoo-Lonhienne et al., 2010a). White et al. (2012) previously reported that bacteria are degraded on surfaces of grass roots through the action of reactive oxygen secreted from root tissues onto bacteria. One of the early effects of surface oxidation of bacteria was release of the nucleic acids from bacterial cells. Nucleic acids diffused from bacteria and adhered to the root hair surface surrounding the lysing bacteria (White et al., 2012). Nucleic acid release from bacteria could be a signal to plant cells to up-regulate proteases and other cellular protein degradation systems. Paungfoo-Lonhienne et al. (2008) also demonstrated that *A. thaliana* possessed proteolytic enzymes that degrade protein on root surfaces and actively engage in endocytosis of protein particles. Beltrán-García et al. (2014) labeled endophytic bacteria with ^{15}N isotope then watered *Agave tequilana* plants with a suspension of viable ^{15}N -labeled bacteria or heat-killed ^{15}N -labeled bacteria and found that plants were able to assimilate ^{15}N -labeled nitrogen from living endophytic bacteria with a much greater efficiency than from heat-killed bacteria. Paungfoo-Lonhienne et al. (2010a,b, 2013) denominated the phenomenon of internalization and degradation of microbes in cells of roots “rhizophagy” or “root eating” to reflect the concept that roots acquire nutrients through a microbivory process.



9.9 THE LYSIS PROCESS

Consistent observation of H_2O_2 activity in and around degrading intracellular bacteria in seedling root tissues suggests that reactive oxygen species (ROS) play an important role in the lysis process. In a study of bacterial lysis on surfaces of seedling roots of *E. arundinaceae* we proposed that secreted ROS and plant proteases functioned to lyse bacteria and their protein contents to provide nitrogenous nutrients, a process we termed “oxidative nitrogen scavenging” to emphasize acquisition of nitrogen from the process (White et al., 2012). The way that we envision this process to function in intracellular bacteria, or bacteria in the periplasmic spaces of cells, is that ROS, including superoxide produced by NADPH oxidases on the plant cell plasma membrane, or other host membranes, is secreted into the vesicles containing bacteria, or into the depressions in the plasma membrane containing bacteria (White et al., 2018). The membranes of plant cells are protected from ROS by sterols that prevent passage of reactive oxygen into the root cell itself (White et al., 2018).

ROS denatures bacterial walls and membranes and enters bacterial cells, damaging proteins and nucleic acids and causing fragmentation of nucleic acids that results in diffusion of nucleic acid fragments from the bacterium into the host cell's cytoplasm (Kocha et al., 1997; Cabiscol et al., 2000). This is consistent with the observations we made on degrading bacteria on surfaces of, and within grass seedling root hairs (White et al., 2012). Nucleic acid fragments may act as signal molecules to stimulate the plant cell to secrete proteolytic enzymes into vacuoles where protein disarticulation is completed (Paungfoo-Lonhienne et al., 2010a). Oligopeptides may then diffuse into the cytoplasm where disarticulation is completed. The basic mechanism for protein degradation is likely to be similar to the autophagy process that is present in all eukaryotes (Xiong et al., 2007). Autophagy is the process whereby eukaryotes degrade their own proteins that have been damaged through oxidation. In plants, autophagy generally occurs in vacuoles (Xiong et al., 2007). The autophagy process seems consistent with our observations in seedling roots where bacteria in vesicles were first oxidized, resulting in enhanced protein staining using aniline blue stain, then a gradual loss of capacity to stain using aniline blue due to degradation of proteins. In our survey (Table 9.1), we frequently observed the fusion of smaller vesicles to form larger vesicles or vacuoles. Ultimately, these bacterial degradation vacuoles may become part of the central vacuole of the plant cell where autophagy in more mature cells generally occurs.



9.10 MICROBIVORY AS A DEFENSE FROM PARASITISM BY ENDOPHYTIC BACTERIA

It seems logical that microbivory in vascular plant roots is a defense mechanism against intracellular invading bacteria. This idea is consistent with our current understanding of how eukaryotic cells employ reactive oxygen as defense against pathogens. In animals, reactive oxygen is involved in the killing and degradation of phagocytic leukocytes (Robinson, 2008). In leukocytes, the killing and degradation by hydrogen peroxide results from the formation of more potent ROS, including hydroxyl radicals, singlet oxygen, and ozone (Robinson, 2008). It is known that plants also secrete reactive oxygen (superoxide) defensively in “oxidative bursts” at sites of pathogen colonization (Lamb and Dixon,

1997). ROS production as a result of pathogen colonization is known to have a direct effect in killing microbial pathogens (Lamb and Dixon, 1997). This model proposes that bacteria are at least weakly pathogenic to seedlings. The invasion of nuclei by bacteria and the potential for negative effects on host nucleic acids seems consistent with this idea.

Current research suggests that microbivory in vascular plant seedlings may function to supplement with nutrients of all types needed for growth and development. This hypothesis demands that we view plants as mixotrophs. In support of this idea, there are an increasing number of studies that suggest that green plants are in fact mixotrophic, simultaneously autotroph and heterotroph. Several species of Orchidaceae and Ericaceae have been shown to receive carbon from mycorrhizal fungi (Tedersoo et al., 2007; Selosse and Roy, 2008). There is evidence that plants secrete proteases and absorb and degrade organic forms of nitrogen, including amino acids, oligopeptides, and proteins (Matsubayashi and Sakagami, 1996; Godlewski and Adamczyk, 2007; Kamarova et al., 2008; Jamtgard et al., 2008; Paungfoo-Lonhienne et al., 2008; Hill et al., 2011) and that plants may consume microbes (Paungfoo-Lonhienne et al., 2010a,b; White et al., 2012, 2017). From an ecological perspective it is also apparent that in some soils in arctic, alpine, and taiga ecosystems the annual plant demand for nitrogen far exceeds the supply of inorganic nitrogen in the soil (Kielland, 1994; Näsholm et al., 2009) and thus organic forms of nitrogen are likely used, or they are converted to inorganic forms to support plant growth. Further, there is the phenomenon of carnivory in plants, including venus flytraps, pitcher plants, and sundews that evolved to capture and degrade insects and small animals (Chia et al., 2004; Galek et al., 1990). These plants have evolved to capture and degrade insects, a relatively complex nutrient source. For plants, lysing bacteria provides a simple nutrient source: Bacterial walls are thin and they are easily degradable rich sources of nutrients.

It seems reasonable to hypothesize that plants obtain some nutrients through microbivory. One of the functions of the plant microbiome may be to provide nutrients to plants. Precisely how important are the nutrients derived from microbivory to fuel plant development has rarely been estimated, but likely depends on the plant species and growth stage of the plant. White et al. (2015) conducted an experiment to estimate rhizobacterial contribution to grass seedling nutrient uptake. In this experiment, White et al. (2015) labeled bacteria with isotopic ^{15}N , extracted their proteins, and incorporated the labeled proteins into agarose. Tall fescue

(*E. arundinaceae*) seedlings with and without their native seed bacteria were grown on the labeled proteins, then shoots were assessed for ^{15}N content. Seedlings bearing the native endophytic rhizobacteria contained approximately 30% more of the labeled nitrogen than those that lacked the seed-transmitted rhizobacteria. This experiment suggests that as much as 30% of the nutrients obtained by plants could come from microbivory- or rhizophagy. The uncertainty in this study and the future need is to determine how much of the nutrients absorbed by plants come directly from degradation of the bacteria, and how much comes from activities of the living bacteria on root surfaces or surrounding roots in liberation of nutrients that may then be absorbed by roots.

Plant phloem-feeding insects of order Homoptera have been shown to possess endosymbiotic microbes (Proteobacteria) within their bodies. These Proteobacteria are intracellular “bacteriocytes” and they multiply and degrade in time to provide proteins and other nutrients for the insects. Multiple species of Proteobacteria are sometimes present within insects where they are referred to as ‘endosymbiotic systems’ (Koga et al., 2013; Sloan and Moran, 2012). Similarly, we hypothesize that the bacteria in plant seedlings constitute “nutritional endosymbiotic systems” of plants that are used as sources of supplemental nutrients at times and in circumstances when sufficient nutrients cannot be extracted from soils.



9.11 THE “RHIZOPHAGY CYCLE” OR “RHIZOPHAGY SYMBIOSIS”

Recent experiments have suggested that plants carry on and within their seeds small communities of bacteria that function in a rhizophagy cycle (White et al., 2017; Irizarry and White, 2017). In the rhizophagy cycle, plants obtain nutrients from bacteria that alternate between an intracellular/endophytic phase and a free-living soil phase (Verma et al. 2017a,b; Prieto et al., 2017; White et al., 2018). Bacteria acquire soil nutrients in the free-living soil phase; nutrients are extracted from bacteria oxidatively in the intracellular/endophytic phase (Verma and White, 2018). In the rhizophagy cycle plants manipulate symbiotic bacteria—using them as transporters of soil nutrients—then induce them to enter into root cell periplasmic spaces at the meristem tip (White et al., 2017; White et al., 2018); here they extract nutrients from bacteria through oxidation/degradation and finally plants

deposit the surviving bacteria, exhausted of their nutrients back into the rhizosphere, exiting from the tips of elongating root hairs (White et al., 2017). The rhizophagy cycle could result in mobilization of many nutrients (organic and inorganic) from soils by bacteria and result in increased nutrient acquisition by plants (Prieto et al., 2017). Considering the close and consistent association of bacteria with plants as endophytes, it is reasonable that plants would develop ways to extract nutrients from symbiotic bacteria (White et al., 2018).



9.12 CONCLUSIONS

Previous research (e.g., Paungfoo-Lonhienne et al., 2010a,b; White et al., 2012, 2017) and our seedling survey (Table 9.1) suggest that rhizophagy is widespread in vascular plants. It is unknown whether some plants rely more heavily than others on rhizophagy to obtain nutrients. Large differences between species and cultivars were observed in the amounts of oxidizing bacteria evident in seedling roots (Table 9.1). Differences were notable in bacterial content of seedling roots of modern white corn, where intracellular bacteria were not observed, and tropical corn, where roots contained abundant intracellular bacteria. These differences could reflect presence or absence of seed-vectored symbiotic bacteria that participate in rhizophagy symbiosis. Presence of bacterial endophytes that participate in rhizophagy symbiosis could explain why tropical corn does not require high fertilizer inputs while modern hybrid corn depends on fertilizer inputs. Crop plants such as *Moringa oleifera* and egusi melon (*Citrullus colocynthis*) that are high in proteins and other nutrients (Juliani et al., 2010; Akubundu et al., 1982) could rely on bacteria involved in rhizophagy symbiosis to scavenge nutrients in soils and carry them back to plants where they may be extracted oxidatively and absorbed into root cells. Because the rhizophagy cycle involves bacteria that are oxidized within roots, plants that are actively involved in a rhizophagy symbiosis not only acquire nutrients but they also may up-regulate genes for oxidative stress resistance and consequently are resistant to abiotic and biotic stresses (White and Torres, 2010). We are only just beginning to recognize rhizophagy symbiosis and many topics need to be addressed. For example, some questions include: Are there nutrient specialists among the symbiotic bacteria that are better at transporting to

plants particular nutrients? How much of the nutrients absorbed by plants comes directly from internal microbe degradation; how much comes from activities of the microbes in the rhizosphere in liberating soil nutrients that are absorbed by roots? Are there bacteria that cause plants to express oxidative stress tolerance but do not carry nutrients? Can we move the seed-vectored symbiotic bacteria between species of plants (Verma and White, 2018) or cultivars and improve rhizophagy cycle activity in crop plants where native symbiotic bacteria have been lost? Precisely what nutrients do plants obtain from rhizophagy symbiosis? Does the plant absorb into root cells partially degraded organic molecules—or are all organic molecules completely oxidized prior to absorption? Do bacteria that colonize root meristems also colonize shoot meristems and thus become distributed to all parts of plants? How do plants induce symbiotic bacteria to enter cells at the root meristem? What are the “signal” molecules that pass between plant and bacterium during the symbiotic interactions? What genes do hosts express during rhizophagy activities? What root cells (superficial layers on root surface or cells deep in the interior of the root axis) are involved in the rhizophagy process? Much additional research will be needed before we will fully understand the rhizophagy process or its ramifications for crop production. Regardless of what is still unknown, it is increasingly clear that rhizophagy symbiosis may represent an important nutritional symbiosis that functions in many or all plants to provide nutrients. Rhizophagy symbiosis may represent a primitive but fundamental process for nutrient acquisition functioning in seedless vascular plants such as ferns as well as seed plants. Understanding how rhizophagy symbiosis functions could lead to new ways to cultivate crops without reliance on excessive agrochemical applications. Finally, learning how to manipulate rhizophagy symbiosis could also result in new technologies for reducing growth of weedy or invasive plant species by inhibiting the symbiosis.

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REFERENCES

- Akubundu, E.N., Cherry, J.P., Simmons, J.G., 1982. Chemical, functional, and nutritional properties of egusi (*Colocynthis citrullus* L.) seed protein products. *J Food Sci*, 47, 829–836.
- Álvarez-Loayza, P., White Jr., J.F., Torres, M.S., Balslev, H., Kristiansen, T., 2011. Light converts endosymbiotic fungus to pathogen, influencing seedling survival and niche-space filling of a common tropical tree, *Iriartea deltoidea*. *PLoS One*, 6 (1), e16386. Available from: <https://doi.org/10.1371/journal.pone.0016386>.
- Alverca, E., Biegala, I., Kennaway, G., Lewis, J., Franca, S., 2002. *In situ* identification and localization of bacteria associated with *Gyrodinium instriatum* (Gymnodiniales, Dinophyceae) by electron and confocal microscopy. *Eur J Phycol*, 37, 523–530.
- Amijee, F., Allan, E.J., Waterhouse, R.N., Glover, L.A., Paton, A.M., 1992. Non-pathogenic association of L-form bacteria (*Pseudomonas syringae* pv. *phaseolicola*) with bean plants (*Phaseolus vulgaris* L.) and its potential for biocontrol of halo blight disease. *Biocontrol Sci Technol*, 2, 203–214.
- Arnold, A.E., Lutzoni, F., 2007. Diversity and host range of foliar fungal endophytes: are tropical leaves biodiversity hotspots? *Ecology*, 88, 541–549.
- Bacon, C.W., Hinton, D.M., 2011. In planta reduction of maize seedling stalk lesions by the bacterial endophyte *Bacillus mojavensis*. *Can J Microbiol*, 57, 1–8.
- Beltrán-García, M.J., White, J.F., Prado, F.M., Prieto, K.R., Yamaguchi, L.F., Torres, M.S., et al., 2014. Nitrogen acquisition in *Agave tequilana* from degradation of endophytic bacteria. *Sci Rep* 4, 6938.
- Beran, V., Havelkova, M., Kaustova, J., Dvorska, L., Pavlik, I., 2006. Cell wall deficient forms of mycobacteria: a review. *Vet Med (Praha)*, 51, 365–389.
- Bierne, H., Cossart, P., 2012. When bacteria target the nucleus: the emerging family of nucleomodulins. *Cell Microbiol*, 14, 622–633.
- Cabiscol, E., Tamarit, J., Ros, J., 2000. Oxidative stress in bacteria and protein damage by reactive oxygen species. *Internat Microbiol*, 3, 3–8.
- Chia, T.F., Aung, H.H., Osipov, A., Goh, N.K., Chia, L.S., 2004. Carnivorous pitcher plant uses free radicals in the digestion of prey. *Redox Rep*, 9, 255–261.
- Clarke, B.B., White Jr., J.F., Hurley, R.H., Torres, M.S., Sun, S., Huff, D.R., 2006. Endophyte-mediated suppression of dollar spot disease in fine fescue. *Plant Dis*, 90, 994–998.
- Clay, K., Holah, J., Rudgers, J.A., 2005. Herbivores cause a rapid increase in hereditary symbiosis and alter plant community composition. *Proc Natl Acad Sci* 102, 12465–12470.
- Compant, S., Reiter, B., Sessitsch, A., Nowak, J., Clement, C., Ait Barka, E., 2005. Endophytic colonization of *Vitis vinifera* L. by plant growth-promoting bacterium *Burkholderia* sp. strain PsJN. *Appl Environ Microbiol*, 71, 1685–1693.
- Compant, S., Clement, C., Sessitsch, A., 2010. Plant growth-promoting bacteria in the rhizo- and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. *Soil Biol Biochem*, 42, 669–678.
- Daulagala, P.W.H.K.P., Allan, E.J., 2003. L-form bacteria of *Pseudomonas syringae* pv. *phaseolicola* induce chitinases and enhance resistance to *Botrytis cinerea* infection in Chinese cabbage. *Physiol Mol Plant Pathol*, 62, 253–263.
- Döbereiner, J., 1992. History and new perspectives of diazotrophs in association with non-leguminous plants. *Symbiosis*, 13, 1–13.

- Döbereiner, J., Baldani, V.L.D., Olivares, F.L., Reis, V.M., 1994. Endophytic diazotrophs: the key to BNF in gramineous plants. In: Hegasi, N.A., Fayez, M., Monib, M. (Eds.), Nitrogen Fixation with Non-legumes. The American University in Cairo Press, Egypt, pp. 395–408.
- Doty, S.L., 2017. Functional importance of the plant microbiome: implications for agriculture, forestry and bioenergy. In: Doty, S.L. (Ed.), Functional Importance of the Plant Microbiome. Springer, Amsterdam, Netherlands, pp. 1–5.
- Frank, A.C., Guzman, J.P.S., Shay, J.E., 2017. Transmission of bacterial endophytes. *Microorganisms* 5, 70. Available from: <https://doi.org/10.3390/microorganisms5040070>.
- Galek, H., Osswald, W.F., Elstner, E.F., 1990. Oxidative protein modification as predigestive mechanism of the carnivorous plant *Dionaea muscipula*: an hypothesis based on in vitro experiments. *Free Radic Biol Med*, 9, 427–434.
- Glick, B.R., 1995. The enhancement of plant growth by free-living bacteria. *Can J Microbiol*, 41, 109–117.
- Godlewski, M., Adamczyk, B., 2007. The ability of plants to secrete proteases by roots. *Plant Physiol Biochem*, 45, 657–664.
- Hamilton, C.E., Gundel, P.E., Helander, M., Saikkonen, K., 2012. Endophytic mediation of reactive oxygen species and antioxidant activity in plants: a review. *Fungal Divers*, 54, 1–10.
- Hill, P.W., Quilliam, R.S., DeLuca, T.H., Farrar, J., Farrell, M., Roberts, P., et al., 2011. Acquisition and assimilation of nitrogen as peptide-bound and D-enantiomers of amino acids by wheat. *PLoS One* 6 (4), e19220. Available from: <https://doi.org/10.1371/journal.pone.0019220>.
- Hurek, T., Reinhold-Hurek, B., Kellenberger, E., Van Montagu, M., 1994. Root colonization and systemic spreading of *Azoarcus* sp. strain BH72 in grasses. *J Bacteriol* 176, 1913–1923.
- Hurek, T., Egener, T., Reinhold-Hurek, B., 1997. Divergence in nitrogenases of *Azoarcus* spp., proteobacteria of beta subclass. *J Bacteriol* 179, 4172–4178.
- Irizarry, I., White, J.F., 2017. Application of bacteria from non-cultivated plants to promote growth, alter root architecture and alleviate salt stress of cotton. *J Appl Microbiol*. Available from: <https://doi.org/10.1111/jam.13414>.
- James, E.K., 2000. Nitrogen fixation in endophytic and associative symbiosis. *Field Crops Res*, 65, 197–209.
- James, E.K., Olivares, F.L., 1998. Infection and colonization of sugar cane and other graminaceous plants by endophytic diazotrophs. *Crit Rev Plant Sci*, 17, 77–119.
- James, E.K., Reis, V.M., Olivares, F.L., Baldani, J.I., Döbereiner, J., 1994. Infection of sugar cane by the nitrogen-fixing bacterium *Acetobacter diazotrophicus*. *J Exp Bot*, 45, 757–766.
- Jamtgard, S., Nasholm, T., Huss-Danell, K., 2008. Characteristics of amino acid uptake in barley. *Plant Soil*, 302, 221–231.
- Johnston-Monje, D., Raizada, M.N., 2011. Conservation and diversity of seed associated endophytes in *Zea* across boundaries of evolution, ethnography and ecology. *PLoS One*, 6 (6), e20396. Available from: <https://doi.org/10.1371/journal.pone.0020396>.
- Juliani, H.R., Fonseca, Y., Acquaya, D., Malumo, H., Malainy, D., Simon, J.E., 2010. Nutritional assessment of moringa (*Moringa* spp.) from Ghana, Senegal and Zambia. *African Natural Plant Products: New Discoveries and Challenges in Chemistry and Quality*. American Chemical Society, Washington, DC, pp. 469–484, Chapter 25.
- Kamarova, N.Y., Thor, K., Gubler, A., Meier, S., Dietrich, D., Weichert, A., et al., 2008. AtPTR1 and AtPTR5 transport dipeptides in planta. *Plant Physiol*, 148, 856–869.
- Kraiser, T., Gras, D.E., Gutierrez, A.G., Gonzalez, B., Gutierrez, R., 2011. A holistic view of nitrogen acquisition in plants. *J Exp Bot*, 62, 1455–1466.
- Kielland, K., 1994. Amino acid absorption by arctic plants: implications for plant nutrition and nitrogen cycling. *Ecology* 75, 2373–2383.

- Kloepper, J.W., 1993. Plant growth-promoting rhizobacteria as biological control agents. In: Metting Jr., F.B. (Ed.), *Soil Microbial Ecology: Applications in Agricultural and Environmental Management*. Marcel Dekker Inc, New York, USA, pp. 255–274.
- Kocha, T., Yamaguchi, M., Ohtaki, H., Fukuda, T., Aoyagi, T., 1997. Hydrogen peroxide-mediated degradation of protein: different oxidation modes of copper- and iron-dependent hydroxyl radicals on the degradation of albumin. *Biochim Biophys Acta*, 1337, 319–326.
- Koga, R., Nikoh, N., Matsuura, Y., Meng, X.-Y., Fukatsu, T., 2013. Mealybugs with distinct endosymbiotic systems living on the same host plant. *FEMS Microbiol Ecol*, 83, 93–100.
- Kovtunovych, G., Lar, O., Kamalova, S., Kordyum, V., Kleiner, D., Kozyrovska, N., 1999. Correlation between pectate lyase activity and ability of diazotrophic *Klebsiella oxytoca* VN 13 to penetrate into plant tissues. *Plant Soil*, 215, 1–6.
- Kuldau, G., Bacon, C.W., 2008. Clavicipitaceous endophytes: their ability to enhance grass resistance to multiple stresses. *Biol Control*, 46, 57–71.
- Lamb, C., Dixon, R.A., 1997. The oxidative burst in plant disease resistance. *Annu Rev Plant Physiol Mol Biol*, 48, 251–275.
- Li, H., Soares, M.A., Torres, M.S., White, J.F., 2015. Endophytic bacterium, *Bacillus amyloliquefaciens*, enhances ornamental hosta resistance to diseases and insect pests. *J Plant Interact*, 10, 224–229.
- Magnani, G., Didonet, C., Cruz, L., Picheth, C., Pedrosa, F., Souza, E., 2010. Diversity of endophytic bacteria in Brazilian sugarcane. *Genet Mol Res*, 9, 258.
- Matsubayashi, Y., Sakagami, Y., 1996. Phytosulfokine, sulfated peptides that induce the proliferation of single mesophyll cells of *Asparagus officinalis* L. *Proc Natl Acad Sci USA*, 93, 7623–7627.
- Mikola, J., 1998. Effects of microbivore species composition and basal resource enrichment on trophic-level biomasses in an experimental microbial-based soil food web. *Oecologia*, 117, 396–403.
- Näsholm, T., Kielland, K., Ganeteg, U., 2009. Uptake of organic nitrogen by plants. *New Phytol*, 182, 31–48.
- Paungfoo-Lonhienne, C., Lonhienne, T.G.A., Rentsch, D., Robinson, N., Christie, M., Webb, R.I., et al., 2008. Plants can use protein as a nitrogen source without assistance from other organisms. *Proc Natl Acad Sci USA*, 105, 4524–4529.
- Paungfoo-Lonhienne, C., Lonhienne, T., Schmidt, S., 2010a. DNA uptake by *Arabidopsis* induces changes in the expression of CLE peptides which control root morphology. *Plant Signal Behav*, 5, 1112–1114.
- Paungfoo-Lonhienne, C., Rentsch, D., Robatzrk, S., Webb, R.I., Sagulenko, E., Nasholm, T., et al., 2010b. Turning the table: plants consume microbes as a source of nutrients. *PLoS One*, 5 (7), e11915. Available from: <https://doi.org/10.1371/journal.pone.0011915>.
- Paungfoo-Lonhienne, C., Visser, J., Lonhienne, T., Schmidt, S., 2012. Past, present and future of organic nutrients. *Plant Soil*, 359, 1–18.
- Paungfoo-Lonhienne, C., Schmidt, S., Webb, R., Lonhienne, T., 2013. Rhizophagy- A new dimension of plant-microbe interactions. In: de Brijn, F.J. (Ed.), *Molecular Microbial Ecology of the Rhizosphere*. Wiley-Blackwell. Pub John Wiley & Sons, Inc, Hoboken, NJ.
- Prieto, K.R., Echaide-Aquino, F., Huerta-Robles, A., Valerio, H.P., Macedo-Raygoza, G., Prado, F.M., et al., 2017. Endophytic bacteria and rare earth elements: promising candidates for nutrient use efficiency in plants. In: Hossain, M., Kamiya, T., Burritt, D., Tram, L.-S.P., Fujiwara, T. (Eds.), *Plant Macronutrient Use Efficiency*. Academic Press, Cambridge, MA, USA, pp. 285–302.

- Puente, M.E., Bashan, Y., 1994. The desert epiphyte *Tillandsia recurvata* harbours the nitrogen-fixing bacterium *Pseudomonas stutzeri*. *Can J Bot*, 72, 406–408.
- Redman, R.S., Sheehan, K.B., Stout, R.G., Rodriguez, R.J., Henson, J.M., 2002. Thermotolerance generated by plant/fungal symbiosis. *Science*, 298, 1581.
- Reinhold-Hurek, B., Hurek, T., 2011. Living inside plants: bacterial endophytes. *Curr Opin Plant Biol*, 14, 435–443.
- Robinson, J.M., 2008. Reactive oxygen species in phagocytic leucocytes. *Histochem Cell Biol*, 130, 281–297.
- Rodríguez, C.E., Mitter, B., Barret, M., Sessitsch, A., Compant, S., 2017. Commentary: seed bacterial inhabitants and their routes of colonization. *Plant Soil*. 422, 129–134. Available from: <https://doi.org/10.1007/s11104-017-3368-9>.
- Rodriguez, R.J., Woodward, C., Kim, Y.O., Redman, R.S., 2009. Habitat-adapted symbiosis as a defense against abiotic and biotic stresses. In: White Jr., J.F., Torres, M.S. (Eds.), *Defensive Mutualism in Microbial Symbiosis*. CRC Press, Boca Raton, FL, USA, pp. 335–346.
- Rosenblueth, M., Martínez-Romero, E., 2006. Bacterial endophytes and their interactions with hosts. *Mol Plant Microbe Interact*, 19, 826–837.
- Sabaneyeva, E.V., Derkacheva, M.E., Benken, K.A., Fokin, S.I., Vainio, S., Skovorodkin, I.N., 2009. Actin-based mechanism of *Holospira obtusa* trafficking in *Paramecium caudatum*. *Protist*, 160, 205–219.
- Selosse, M.-A., Roy, M., 2008. Green plants that feed on fungi. *Trends Plant Sci*, 14, 64–70.
- Sloan, D.B., Moran, N.A., 2012. Endosymbiotic bacteria as a source of carotenoids in whiteflies. *Biol Lett*. 8, 986–989. Available from: <https://doi.org/10.1098/rsbl.2012.0664>.
- Soares, M.A., Li, H., Bergen, M., White, J.F., 2015. Functional role of an endophytic *Bacillus amyloliquefaciens* in enhancing growth and disease protection of invasive English ivy (*Hedera helix* L.). *Plant Soil*. 405, 107–123. Available from: <https://doi.org/10.1007/s11104-015-2638-7>.
- Soares, M.A., Li, H.Y., Kowalski, K.P., Bergen, M., Torres, M.S., White, J.F., 2016. Functional roles of bacteria from invasive *Phragmites australis* in promotion of host growth. *Microb Ecol*, 72 (2), 407–417.
- Stone, J.K., Bacon, C.W., White Jr., J.F., 2000. An overview of endophytic microbes: endophytism defined. In: Bacon, C.W., White Jr., J.F. (Eds.), *Microbial Endophytes*. Marcel-Dekker, New York, USA, pp. 3–30.
- Taulé, C., Marenque, C., Barlocco, C., Hackembruch, F., Reis, V.M., Sicardi, M., et al., 2012. The contribution of nitrogen fixation to sugarcane (*Saccharum officinarum* L.), and the identification and characterization of part of the associated diazotrophic bacterial community. *Plant Soil*, 356, 35–49.
- Tedersoo, L., Pellet, P., Koljalg, U., Selosse, M., 2007. Parallel evolutionary paths to mycoheterotrophy in understorey Ericaceae and Orchidaceae: ecological evidence for mixotrophy in Pyroleae. *Oecologia*, 151, 206–217.
- Thomas, P., Soly, T.A., 2009. Endophytic bacteria associated with growing shoot tips of banana (*Musa* sp.) cv. Grand Naine and the affinity of endophytes to the host. *Microb Ecol*, 58, 953–964.
- Thomas, P., Reddy, K.M., 2013. Microscopic elicitation of abundant endophytic bacteria colonizing the cell wall-plasma membrane peri-space in the shoot-tip tissue of banana. *AoB PLANTS* 5, plt011. Available from: <https://doi.org/10.1093/aobpla/plt011>.
- Torres, M.S., White, J.F., Zhang, X., Hinton, D.M., Bacon, C.W., 2012. Endophyte-mediated adjustments in host morphology and physiology and effects on host fitness traits in grasses. *Fungal Ecol*, 5, 322–330.

- Urguiaga, S., Cruz, K., Boddey, R., 1992. Contribution of nitrogen fixation to sugar cane: nitrogen-15 and nitrogen balance estimates. *Soil Sci Soc Am J*, 56, 105–114.
- Verma, S.K., Kingsley, K., Irizarry, I., Bergen, M., Kharwar, R.N., White, J.F., 2017a. Seed vectored endophytic bacteria modulate development of rice seedlings. *J Appl Microbiol*, 122, 1680–1691.
- Verma, S.K., Kingsley, K., Bergen, M., English, C., Elmore, M., Kharwar, R.N., et al., 2017b. Bacterial endophytes from rice cut grass (*Leersia oryzoides* L.) increase growth, promote root gravitropic response, stimulate root hair formation, and protect rice seedlings from disease. *Plant Soil*. 422, 223–238. Available from: <https://doi.org/10.1007/s11104-017-3339-1>.
- Verma, S.K., White, J.F., 2018. Indigenous endophytic seed bacteria promote seedling development and defend against fungal disease in browntop millet (*Urochloa ramosa* L.). *J Appl Microbiol*. 124, 764–778. Available from: <https://doi.org/10.1111/jam.13673>.
- Wall, S., Kunze, Z.M., Saboor, S., Soufleri, I., Seechurn, P., Chiodini, R., et al., 1993. Identification of spheroplast-like agents isolated from tissues of patients with Crohn's disease and control tissues by polymerase chain reaction. *J Clin Microbiol*, 31, 1241–1245.
- Waller, F., Achatz, B., Baltruschat, H., Fodor, J., Becker, K., Fisher, M., et al., 2005. The endophytic fungus *Piriformospora indica* reprograms barley to salt-stress tolerance, disease resistance, and higher yield. *Proc Natl Acad Sci* 102, 13386–13391.
- Weber, O.B., Muniz, C.R., Vitor, A.O., Freire, F.C.O., Oliveira, V.M., 2007. Interaction of endophytic diazotrophic bacteria and *Fusarium oxysporum* f. sp. *cubense* on plantlets of banana 'Maca'. *Plant Soil*, 298, 47–56.
- White, J.F., Torres, M.S., 2010. Is endophyte-mediated defensive mutualism oxidative stress protection?. *Physiol Plant*, 138, 440–446.
- White, J.F., Crawford, H., Torres, M.S., Mattera, R., Irizarry, I., Bergen, M., 2012. A proposed mechanism for nitrogen acquisition by grass seedlings through oxidation of symbiotic bacteria. *Symbiosis* 57 (3), 161–171. Available from: <http://doi.org/10.1007/s13199-012-0189-8>.
- White, J.F., Torres, M.S., Somu, M.P., Johnson, H., Irizarry, I., Chen, Q., et al., 2014. Hydrogen peroxide staining to visualize intracellular bacterial infections of seedling root cells. *Microsc Res Tech* 77 (8), 566–573. Available from: <https://doi.org/10.1002/jemt.22375>.
- White, J.F., Chen, Q., Torres, M.S., Mattera, R., Irizarry, I., Tadych, M., et al., 2015. Collaboration between grass seedlings and rhizobacteria to scavenge organic nitrogen in soils. *AoB Plants* 7, plu093. Available from: <https://doi.org/10.1093/aobpla/plu093>.
- White, J.F., Kingsley, K.I., Kowalski, K.P., Irizarry, I., Micci, A., Soares, M.A., et al., 2017. Disease protection and allelopathic interactions of seed-transmitted endophytic pseudomonads of invasive seed grass (*Phragmites australis*). *Plant Soil* 422, 195–208. Available from: <https://doi.org/10.1007/s11104-016-3169-6>.
- White, J.F., Kingsley, K., Harper, C.J., Verma, S.K., Brindisi, L., Chen, Q., et al., 2018. Reactive oxygen defense against cellular endoparasites and the origin of eukaryotes. In: Krings, M., Harper, C.J., Cuneo, N.R., Rothwell, G.W. (Eds.), *Transformative Paleobotany: Papers to Commemorate the Life and Legacy of Thomas N. Taylor*. Elsevier, Amsterdam, Netherlands.
- Xiong, Y., Contento, A.L., Nguyen, P.Q., Basham, D.C., 2007. Degradation of oxidized proteins by autophagy during oxidative stress in *Arabidopsis*. *Plant Physiol*, 143, 291–299.

FURTHER READING

- Baker, G.C., Smith, J.J., Cowan, D.A., 2003. Review and re-analysis of domain-specific 16S primers. *J Microb Methods*, 55, 541–555.
- Kowalski, K.P., Bacon, C., Bickford, W., Braun, H., Clay, K., Leduc-Lapierre, M., et al., 2015. Advancing the science of microbial symbiosis to support invasive species management: a case study on *Phragmites* in the Great Lakes. *Front Microbiol* 6, 95. Available from: <https://doi.org/10.3389/fmicb.2015.00095>.
- Munkres, K.D., 1990. Histochemical detection of superoxide radicals and hydrogen peroxide by *Age-1* mutants of *Neurospora*. *Fungal Genet Newslett*, 37, 24–25.
- Pick, E., Keisari, Y., 1980. A simple colorimetric method for the measurement of hydrogen peroxide produced by cells in culture. *J Microbiol Methods*, 38, 161–170.
- Radajewski, S., Ineson, P., Parekh, N., Murrell, J.C., 2000. Stable-isotope probing as a tool in microbial ecology. *Nature*, 403, 646–649.

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Portraying Rhizobacterial Mechanisms in Drought Tolerance: A Way Forward Toward Sustainable Agriculture

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10.1 INTRODUCTION

It is estimated that global population will be around 8–9 billion by 2030 necessitating increased crop productivity to ensure food security while also promoting sustainable and resilient agriculture. Drought is among dominant abiotic stressors hindering crop growth thus leading to significant losses in agricultural productivity (Mahalingam, 2015). Water deficit in soil cause by drought decreases soil water potential resulting in cell dehydration finally causing inhibited cell expansion and cell division, thus resulting in osmotic stress. Moreover, it results in oxidative stress due to production of reactive oxygen species (ROS) in plants (Vurukonda et al., 2016). Plants have been blessed with an array of intrinsic metabolic mechanisms to continuously cope up with the rapid and adverse stressed conditions (Simontacchi et al., 2015). The alterations in soil water content due to drought disturbs the plant homeostasis thus creating necessity for the plant to harbor some advanced genetic and metabolic mechanisms within its cellular system. Moreover, inclusion of rhizobacteria popularly known as PGPR (plant growth promoting rhizobacteria) in agriculture can lessen the burden on plants internal machinery due to environmental stress (Ngumbi and Kloepper, 2014). The intrinsic metabolic potential of rhizobacteria can be harnessed to improve metabolic capability of the plants to combat drought stresses (Nguyen et al., 2016). This review is an attempt to highlight various stress tolerance mechanisms elucidated by

rhizobacteria to enhance plant growth and productivity during drought stress.



10.2 RHIZOBACTERIAL MEDIATED MECHANISMS OF DROUGHT STRESS TOLERANCE

Kaushal and Wani (2015) have coined term RIDER (rhizobacterial-induced drought endurance and resilience) that encompasses an array of mechanisms induced by rhizobacteria causing various morphological, physiological, and biochemical alterations that can help plants to combat drought stress in plants (Fig. 10.1). It comprises changes in phytohormonal content, antioxidant defense, production of osmoprotectants, and exopolysaccharides (EPS). Moreover, it includes production of stress responsive genes and volatile organic compounds (VOCs). Synthesis of phytohormones by rhizobacterial strains changes the hormonal content of plant hormones that stimulate plant growth during biotic stresses (Table 10.1). Water deficit conditions caused as a result of drought negatively impact the root growth which in turn affects the plant growth. Thus, to overcome the growth limitations caused as an impact on roots,

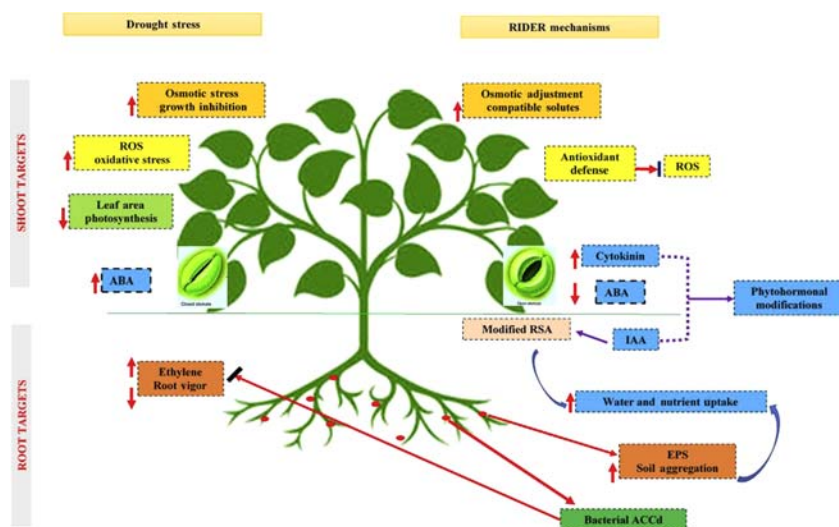


Figure 10.1 Different RIDER mechanisms exhibited by rhizobacteria to combat drought stress in plants.

Table 10.1 RIDER mechanisms imparting drought tolerance in plants

PGPR	Plant species	Possible RIDER mechanism	Reference
<i>Azospirillum brasilense</i>	Arabidopsis	Relative water content, ABA, Stomatal conductance, Lipid peroxidation	Cohen et al. (2015)
<i>Achromobacter xylosoxidans</i>	Sunflower	Phytohormones—ABA, SA and JA	Castillo et al. (2013)
<i>Achromobacter piechaudii</i>	Tomato, Peppers	Ethylene level, Relative water content	Mayak et al. (2004)
<i>A. brasilense</i>	Tomato	IAA induced pathway enhanced root hair development	Molina-Favero et al. (2008)
<i>Azospirillum lipoferum</i>	Wheat	Water potential and IAA enhanced root growth	Arzanesh et al. (2011)
<i>A. lipoferum</i>	Maize	Relative water content, Gibberellins increased ABA level	Bano et al. (2013)
<i>Bacillus licheniformis</i>	Pepper	Stress-related genes including Cadhn, VA, sHSP, CaPR10	Lim and Kim (2013)
<i>Bacillus cereus</i>	Cucumber	Proline content, Root vigor, Chlorophyll content	Wang et al. (2012)
<i>Bacillus pumilus</i>	Potato	ROS	Gururani et al. (2013)
<i>Bacillus</i> spp.	Sorghum	Proline level, Sugar content	Grover et al. (2014)
<i>Bacillus amyloliquefaciens</i>	Wheat	Expression of stress genes APX1, SAMS1, and HSP17.8	Kasim et al. (2013)
<i>Bacillus. thuringiensis</i>	Wheat	Root adhering soil, VOC	Timmusk et al. (2014)
<i>Burkholderia phytofirmans</i>	Maize	Membrane permeability, shoot fresh and dry weight	Naveed et al. (2014)
<i>Burkholderia</i> spp.	Maize	ROS, lipid peroxidation	Fan et al. (2015)
<i>Bacillus polymyxa</i>	Tomato	Proline accumulation	Shintu and Jayaram (2015)
<i>Bacillus</i> spp.	Lettuce	Cytokinin	Arkipova et al. (2007)
<i>Bacillus cereus</i>	Barley	ACC deaminase	Timmusk et al. (2011)
<i>Gluconacetobacter diazotrophicus</i>	Sugarcane	ABA-dependent signaling genes	Vargas et al. (2014)
<i>Klebsiella variicola</i>	Maize	Accumulation of choline	Gou et al. (2015)
<i>Paenibacillus polymyxa</i>	Arabidopsis	Expression of stress inducing genes RAB18, LT178, and ERD15	Timmusk and Wagner (1999)

(Continued)

Table 10.1 (Continued)

PGPR	Plant species	Possible RIDER mechanism	Reference
<i>Phyllobacterium brassicacearum</i>	Arabidopsis	Sucrose and ABA contents	Bresson et al. (2013)
<i>Pseudomonas</i> spp.	Pea	Root architecture, Chlorophyll content	Arshad et al. (2008)
<i>Pseudomonas fluorescens</i>	Maize	Proline content, ABA	Ansary et al. (2012)
<i>Pseudomonas aeruginosa</i>	Mung bean	Up regulation of drought stress genes DREB2A, CAT1, DHN	Sarma and Saikia (2014)
<i>Proteus penneri</i>	Maize	Relative water content, proline content	Naseem and Bano (2014)
<i>Pseudomonas putida</i>	Soybean	Secretion of gibberellins	Sang-Mo et al. (2014)
<i>Pseudomonas chlororaphis</i>	Arabidopsis	JA, SA, and Ethylene-response gene	Cho et al. (2013)
<i>Rhizobium etli</i>	Beans	Trehalose	Reina-Bueno et al. (2012)
<i>Rhizobium leguminosarum</i>	Wheat	IAA	Hussain et al. (2014)
<i>Serratia</i> spp.	Wheat	ACC deaminase	Bangash et al. (2013)
<i>Sinorhizobium meliloti</i>	Alfalfa	Cytokinin	Xu et al. (2012)
<i>V. paradoxus</i>	Pea	ABA	Belimov et al. (2009)

alterations in the root system architecture (RSA) can be exploited as a stress defensive mechanism (Postma and Lynch, 2011). The basic adaptive mechanism of rhizobacterial inoculation to enhance plant growth during water deficit conditions includes their ability to alter the RSA which can raise the number of root tips and root surface area (Vacheron et al., 2013) ultimately enhancing water and nutrient absorption. *Alcaligenes faecalis* inoculated maize plants displayed increased root length by 10% in comparison to control that enhanced water uptake thus conferring drought tolerance to plants. Similar observations were reported in maize plants inoculated with *Burkholderia phytofirmans* strain. A significant increase in root biomass by 70% and 58% was observed in Mazurka and Kaleo cultivars, respectively. Timmusk et al. (2014) reported rhizobacterial mediated modifications in root architecture which enhanced drought stress tolerance in plants. *Bacillus thuringiensis* inoculated wheat plants showed

increased lateral root density and length as well as root hair density and length (59% and 200%), respectively in response to IAA. Inoculation of plants under drought stress leads to enhanced shoot growth. Timmusk et al. (2014) observed 78% higher biomass in *B. thuringiensis* inoculated wheat plants under drought stress in comparison to nontreated plants. Relative water content (RWC) in plant leaves is a major physiological parameter to measure plant water status as it is involved in the metabolic activities. Increased RWC is correlated to enhanced drought tolerance as it helps plants to counteract the oxidative and osmotic stresses caused by drought stress (Vardharajula et al., 2011; Bano et al., 2013).



10.3 MODULATIONS IN PHYTOHORMONAL LEVELS

Plant hormones are central connecting links that play a role in reprogramming the developmental and major signaling cascades involved in stress tolerance. Elevation in shoot and root biomass was noticed in clover (*Trifolium repens* L.) plants inoculated with PGPR (*Pseudomonas putida* and *Bacillus megaterium*) which was positively correlated to enhanced IAA levels thus increasing plant growth and endurance during drought stress (Marulanda et al., 2009). *Pseudomonas chlororaphis* TSAU13 treated tomato and cucumber plants displayed higher root growth due to IAA production resulting in improved conductivity of water in comparison to control plants under drought conditions (Egamberdieva and Kucharova, 2009). An up regulation of indole-3-pyruvate decarboxylase gene was noticed in *Azospirillum* treated wheat seedlings causing an elevation in IAA synthesis. This led to architectural modification in coleoptiles xylem basically enlarged xylem vessels that raised water uptake in inoculated plants (Pereyra et al., 2012). It was noticed that IAA production led to higher lateral root density and length in *B. thuringiensis* inoculated wheat plants during drought (Timmusk et al., 2014). An increase in specific root length and root area was noticed in *Azospirillum brasilense* inoculated common bean plants during drought. These alterations in root morphology can be linked to ability of *A. brasilense* to form auxins (Dimkpa et al., 2009). Similar observations were recorded in wheat plants inoculated with *Azospirillum*. IAA production increased water level and decreased water potential in leaves. IAA increased root growth and development of

lateral roots which led to higher water uptake in plants (Arzanesh et al., 2011). The physiological and metabolic status of plant was upgraded as a result of IAA production in *B. thuringiensis* treated *Lavandula* plants that helped them to survive during drought stress (Armada et al., 2014). The gibberellins (GAs) regulate various plant process such as germination of seeds, stem elongation, flowering, fruit ripening, and leaf and fruit senescence (Daviere and Achard, 2013). Inoculation of soybean plants with GAs producing PGPR *P. putida* H-2-3 enhanced plant growth during drought stress (Kang et al., 2014b). *Azospirillum lipoferum* treated maize plants survived under drought due to production of GAs (Cohen et al., 2009). Enhanced plant growth was observed as a result of raised endogenous content of GAs in PGPR (*Burkholderia cepacia* SE4, *Promicromonospora* spp. SE188 and *Acinetobacter calcoaceticus* SE370) treated cucumber plants in comparison to uninoculated plants during drought and salinity stress (Kang et al., 2014a). Soybean plants inoculated with GAs secreting PGPR, *P. putida* H-2-3 helped plants to combat drought stress (Kang et al., 2014b). Synthesis of GA in *Azospirillum* inoculated wheat plants helped them to survive under drought stress (Creus et al., 2005). Cytokinins enhance cytokinesis in roots and shoots and regulate stomatal opening. They also play important role in apical dominance and inhibits leaf senescence. Various research studies have revealed positive effects on plant growth after inoculation with cytokinin producing rhizobacteria during stress conditions. Lettuce plants on inoculation with a cytokinin forming PGPR *Bacillus subtilis* showed enhanced shoot biomass and reduction of the root to shoot ratio (Arkhipova et al., 2007) concluding root-to-shoot cytokinin signaling. Increase in root and shoot biomass was observed in maize plants inoculated with cytokinin synthesizing *Micrococcus luteus* chp37 (Raza and Faisal, 2013). *Platykladeus orientalis* container seedlings treated with *B. subtilis* displayed stimulated root biomass due to increased cytokinin levels by 47.52% in leaves that helped plants to cope drought stress. Moreover, the damage such as stomatal opening caused as a result of elevation in cytokinin levels was counteracted by increased abscisic acid (ABA) level (Liu et al., 2013). Enhanced plant growth was noticed as a result of ABA synthesis in grapevine plants inoculated with *Bacillus licheniformis* and *Pseudomonas fluorescens* under water stress conditions (Salomon et al., 2014). ABA synthesis is accelerated during water deficit conditions created by drought stress resulting in stomatal closure to lessen transpirational water loss, enhance root branching to improve water uptake in plants (Weiner et al., 2010). Jasmonic acid (JA)

and salicylic acid (SA) also has pivotal role in protection of plants from damages caused by oxidative stress as a result of drought (Iqbal and Ashraf, 2010). SA-producing *Achromobacter xylosoxidans* and *Bacillus pumilus* inoculated sunflower seedlings displayed increased biomass of sunflower seedlings under drought stress (Forchetti et al., 2010). Higher ABA levels were recorded in *A. lipoferum* inoculated maize plants (Cohen et al., 2009). In another study *A. brasilense* treated *Arabidopsis* plants displayed alterations in root architecture involving higher lateral roots, stimulation of photosynthetic pigments, and decreased water loss through reduced stomatal conductance during drought stress (Cohen et al., 2015). In contrast to this, Kang et al. (2014a) observed decrease in ABA level in cucumber plants inoculated with PGPR (*B. cepacia* SE4, *Promicromonospora* spp. SE188 and *A. calcoaceticus* SE370). Similar observations of ABA reduction were recorded in *P. putida* H-2-3 inoculated soybean plants in comparison to inoculated plants. However, elevation in JA and SA content was observed (Kang et al., 2014b). Aroca et al. (2006) correlated the ABA enhanced drought tolerance in plants by regulating transpiration mechanism in leaves, root hydraulic conductivity, or through the up regulation of aquaporins (Zhou et al., 2012). It would be enthralling to unveil the role of rhizobacterial mediated alterations in ABA levels to notice drought tolerance in plants. Synthesis of ethylene increases during drought hence negatively affecting root development and affects plant growth. It was observed that ACC which is precursor of ethylene formed by the plants in roots is released in the rhizosphere, which then enters rhizobacterial strains that possess ACC deaminase (ACCD). Various studies have revealed the potential of ACCd producing PGPR under drought stress. ACCd hydrolyzes ACC to ammonia and α -ketobutyrate, thus reducing ethylene content (Glick, 2014). Increase in fresh and dry weight was noticed in tomato and pepper plants on inoculated with *Achromobacter piechaudii* ARV8 having ACCd. This was related to the reduction in ethylene levels that helped plant to survive drought stress (Mayak et al., 2004). Inoculating *Pisum* plants with PGPR *Pseudomonas* having ACCd activity displayed root elongation which raised uptake of water thus ensuring plant growth and survival during water stress (Zahir et al., 2008). Treatment of *Pisum* with *Variovorax paradoxus* 5C-2 reduced xylem ACC content in drying soils hence lowering synthesis of shoot ethylene thus alleviating drought stimulated reduction in nodule formation and nitrogen seed content (Belimov et al., 2009). Pepper plants on inoculation with *B. licheniformis* K11 possessing ACCd activity enhanced plant growth due

to reduced ethylene content by ACCd activity (Lim and Kim, 2013). Wang et al. (2005) noticed that Arabidopsis plants inoculated with *P. fluorescens* FPT9601-T5 resulted in up regulation of auxin related genes. In contrast, ethylene responsive genes were down regulated during biotic stress. Enhanced root growth in rhizobacterial inoculated plants observed due to raised IAA synthesis and reduction in ethylene content is attributed to linkage among IAA and ethylene precursor ACC (Lugtenberg and Kamilova, 2009).



10.4 OSMOLYTE PRODUCTION TO REDUCE OSMOTIC STRESS

Accumulation of osmolyte or solute content per cell for maintaining the cell turgor and normal cellular physiological functions known as osmotic adjustment is a widely renowned physiological adaptive mechanism in plants that alleviates osmotic stress (Gill and Tuteja, 2010). Synthesis of compatible solutes by PGPR strains and plants in response to osmotic stress, acts collaboratively to enhance plant growth and survival (Paul and Nair, 2008). Presence of substrates in rhizospheric soil and time of osmotic stress are key factors that play important role in synthesis of a defined solute in rhizobacteria during drought stress. Synthesis of compatible solutes maintains necessary cellular turgor and reduces water potential in plants without lowering actual water content. Proline is among major osmolytes that is synthesized and accumulated as a result of protein hydrolysis to combat drought stress (Huang et al., 2013). In addition, proline has multifarious roles such as adjustment of cytosolic acidity, minimizing lipid peroxidation through free radical scavenging, and stabilization of sub cellular structures such as proteins and membranes (Hayat et al., 2012). Plants treated with rhizobacteria display higher proline levels in response to osmotic stress as result of drought (Vardharajula et al., 2011; Bharti et al., 2014; Sarma and Saikia, 2014) however it is still doubtful that whether this enhanced content is due to increased absorption from rhizosphere or through an up regulation of proline biosynthetic pathway. It was observed that maize plants inoculated with PGPR *P. putida* GAP-P45 showed raised proline accumulation which in turn enhanced plant biomass and improved relative leaf water potential (Vardharajula et al., 2010). Ansary et al. (2012) observed that PGPR *P. fluorescens* inoculated maize

plants showed an increase in proline content which in addition to already existed proline levels helped plants to alleviate drought stress. An identical adaptive drought mechanism was observed in *B. thuringiensis* treated *Lavandula* plants which were attributed to increased accumulation of proline in shoots proline in comparison to control plants (Armada et al., 2014). It was observed that inoculation of drought tolerant and drought sensitive cultivars of rice (*Oryza sativa* L.) with a consortium of PGPR strains comprising *Pseudomonas jessenii* R62, *Pseudomonas synxantha* R81, and *Arthrobacter nitroguajacolicus* strain YB3, strain YB5 increased proline accumulation in plants (Gusain et al., 2015). Similar observations have been recorded in *Zea mays* plants treated with PGPR *P. putida* GAP-P45 (Vardharajula et al., 2010) and *A. lipoferum* (Bano et al., 2013) enhanced plant growth via accumulation of free amino acids in comparison to untreated plants during drought stress. Increase in proline synthesis was observed in (*Lycopersicon esculentum* Mill) cv. *Anakha* plants inoculated with phosphate solubilizing bacteria *Bacillus polymyxa* that helped plants to survive during drought stress (Shintu and Jayaram, 2015). Starch hydrolysis resulted in higher accrual of soluble sugars in rhizobacterial treated maize seedlings which negated drought stress effects on plants (Bano and Fatima, 2009; Vardharajula et al., 2011). Biosynthesis of trehalose also has major contribution toward osmotolerance in plants inoculated with rhizobacteria as it imparts stabilization to membranes and proteins. (Yang et al., 2010). Plants inoculated with rhizobacterial strains survived drought stress due to raised osmolyte contents, as genetically engineered rhizobacteria were capable of overexpressing trehalose biosynthetic genes. It was observed that trehalose synthesis was raised in *Rhizobium etli* (overexpressing trehalose-6-phosphate synthase) treated common bean plants in contrast to plants treated with wild type strain (Suarez et al., 2008). Similar observations of enhanced trehalose content were observed in maize plants inoculated with *A. brasilense* that showed overexpression of trehalose biosynthetic genes (Rodriguez et al., 2009). Glycine betaine such as quaternary compounds are known for increasing stress tolerance in plants as it imparts stabilization to membranes and RuBisCO activity (Chen and Murata, 2008). Inoculation of *Arabidopsis* plants with *B. subtilis* GB03 showed raised glycine betaine and choline content (Zhang et al., 2010). Wang et al. (2012) observed enhanced proline contents in cucumber plants treated with a consortium of three rhizobacterial strains called BBS (*Bacillus cereus* AR156, *B. subtilis* SM21, and *Serratia* spp. XY21) in contrast to untreated controls that contributed toward RIDER. Choline is a

primary metabolite involved in synthesis glycine betaine (GB) which plays a crucial role in stress tolerance (Zhang et al., 2010). GB protects the plant from adverse effects during drought stress via maintaining osmotic balance and protection of extrinsic proteins in photosystem II. Research studies have revealed that inoculation of *Arabidopsis* by *B. subtilis* GB03 and *Zea mays* by *Klebsiella variicola* F2, *P. fluorescens* YX2, and *Raoultella planticola* YL2 enhanced choline biosynthesis. This in turn caused accumulation of GB that improved RWC in leaves and dry matter weight in plants (Glick et al., 2007; Zhang et al., 2010; Gou et al., 2015). It was observed that *Arabidopsis* plants inoculated with *B. subtilis* (GB03) displayed elevation in endogenous Cho and GB metabolite content by more than two- and fivefold, respectively. Polyamines are organic cations possessing aliphatic nitrogen structure that plays pivotal role in various physiological and basic cellular processes such as root elongation, membrane stabilization, programmed cell death (PCD), replication, transcription, and translation (Gupta et al., 2013). Accumulation of polyamines during stress have been correlated to enhanced plant growth. Inoculation of *Oryza* seedlings with *A. brasilense* Az39 showed higher root growth was observed as a result of rhizobacterial cadaverine (polyamine) production which stimulated plant growth (Cassan et al., 2009). *Arabidopsis* inoculation with *B. megaterium* BOFC15 showed elevation in accumulation of polyamine which resulted in activation of increased PA-mediated signaling pathways that alleviated drought stress by maintaining water levels and elevated photosynthetic activity (Zhou et al., 2016).



10.5 ANTIOXIDANT DEFENSIVE MACHINERY

Normally, the generation of ROS as by-products as a result of aerobic metabolism in plant cells is low in different plant organelles. However, when plants are exposed to drought stress overproduction of ROS alters the redox homeostasis of plant cells. Increased ROS content creates oxidative stress that hinders the metabolic activity ultimately leading to PCD. Hence, oxidative stress can be defined as production of ROS above threshold pool which creates imbalance in redox homeostasis of cell. Higher ROS content causes protein oxidation, oxidative damage to DNA and reduces fluidity of membranes. Other damages include inhibition of

protein synthesis and loss of enzymatic activities. During drought, stress lipids are hotspots for ROS damage. Polyunsaturated fatty acids (PUFA) major components of plasma membrane are attacked by ROS which initiates lipid peroxidation that aggravates the oxidative stress. This causes degradation of fatty acids resulting in production of various products such as aldehydes. Plants are armed with effective antioxidant defensive machinery comprising enzymatic and nonenzymatic components to mitigate the drought induced detrimental effects of ROS (Miller et al., 2010). Major enzymes of antioxidant machinery consist of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), glutathione reductase (GR). Ascorbate (AsA), glutathione (GSH), cysteine, glutathione, tocopherols are nonenzymatic components of antioxidant defense. It is well known fact that ROS at higher levels causes detrimental effects however research studies have established it as a signaling molecule which is involved in various stress responsive and defensive pathways (Baxter et al., 2014). Therefore, it is essential for plants to sustain precise equilibrium between ROS generation and ROS quenching systems to alleviate oxidative damage and manage signaling pathways. Numerous studies have reported that rhizobacterial inoculated plants show elevated levels of antioxidant enzymes which contribute toward plant growth and survival during oxidative stress through manipulation of antioxidant enzymes. Treatment of cucumber plants with BBS consortium, induced an increase in the activity of SOD that enhanced drought tolerance (Wang et al., 2012). Enhanced plant growth was observed in PGPR inoculated tomato plants which were attributed to increased levels of APX, SOD, and CAT. In addition, a significant increase in the specific activity of CAT (1.8 times) was observed in inoculated plants in comparison to control plants (Gururani et al., 2013). Enhanced activity of CAT was recorded in green gram plants inoculated with *P. fluorescens* Pfl and *B. subtilis* EPB which established correlation with the increased drought endurance. Similar observations were recorded in mung bean plants treated with *Pseudomonas* strain GGRJ21 during drought stress, there was an increase in activity of CAT, peroxidase (POX), and SOD. However, CAT activity was noticed to raise over 15–36 days and then reduced; however, activities of POX and SOD were found to increase over 15–43 days (Sarma and Saikia, 2014). Increased activity of CAT was noticed in *Ocimum basilicum* L. inoculated with *Pseudomonas*. Similarly, treatment of *Ocimum basilicum* L with a consortium of *Pseudomonads* sp., *Bacillus lentus*,

and *A. brasilense* showed enhanced activity of glutathione peroxidase (GPX) and APX (Heidari and Golpayegani, 2011). Inoculation of Sahbhagi (drought tolerance) and IR-64 (drought sensitive) cultivars of rice (*O. sativa* L.) with a consortium of *P. jessenii* R62, *P. synxantha* R81 and *A. nitroguajacolicus* strain YB3 and strain YB5 induced activity of SOD, CAT, and APX under drought stress in comparison to control plants during drought stress (Gusain et al., 2015). However contrary results were observed in maize plants treated with *P. putida* GAP-P45 that displayed reduced levels APX, CAT, and GPX thus establishing that rhizobacterial enhanced drought tolerance in inoculated in comparison to uninoculated plants (Vardharajula et al., 2010). A similar drought tolerant response was observed in *Lavandula* plants treated with *B. thuringiensis*. Decreased activities of GR and APX activity were correlated promoted plant growth during drought (Armada et al., 2014). It was recorded that activities of CAT, POX, polyphenol oxidase decreased in PGPR treated cucumber plants as compared to control which abated drought stress (Kang et al., 2014a). Hence rhizobacterial role through antioxidant machinery in alleviating drought stress is well established; however, there is still some uncertainty regarding the levels of antioxidant enzymes. These alterations in enzymatic levels depend on numerous factors such as type of host plant, rhizobacterial strain, and time period of stress. Research studies should be focused to unveil ROS cross talks with phytohormones during stress conditions.



10.6 RHIZOBACTERIAL EXOPOLYSACCHARIDES PRODUCTION

Rhizobacterial EPS containing homo or hetero polysaccharides are released into soil as capsule or in slime form. The acyl group provides EPS an anionic form that enhances its lipophilic nature ultimately influencing its cooperative interactions with cations through hydrogen bonding, Vander Waals forces and adsorption mechanisms (Davey and O'Toole, 2000). Various factors such as bacterial growth phase, medium composition, and environmental conditions are responsible for EPS composition and production. A smart hydrated microenvironment is created by EPS production which possesses water holding capacity and dries up more slowly in comparison to neighboring environment hence,

protecting the plant roots from drying effects of drought. It plays a pivotal role in the improving soil permeability through formation and stabilization of soil micro and macroaggregates which increase nutrient and water uptake across plant brings thus enhancing plant growth (Selvakumar et al., 2012). EPS production by rhizobacterial strains plays important role in promoting plant growth during water through formation of hydrophilic biofilms around plant roots conferring protection against desiccation (Rossi et al., 2012). Water holding capacity of rhizobacterial strains depends on polysaccharide contents of EPS (Vu et al., 2009). It was observed that improved soil structure and aggregation properties increased plants adaptation to water stress through EPS produced by *Azospirillum* (Bashan et al., 2014). A marked increase in dry biomass, nitrogen nutrition, and water uptake in response to increased root adhering soil per root tissue (RAS/RT) ratio and RAS macroporosity was recorded in sunflower rhizosphere treated with EPS-producing *Rhizobium* spp. strain YAS34 during drought stress (Alami et al., 2000). Amendments in soil aggregation were observed in rhizosphere of wheat seedlings because of EPS production by rhizobia hence promoting plant growth during stress (Kaci et al., 2005). A significant increase RAS/RT ratio and macroaggregate stability was observed in plants inoculated with EPS producing drought tolerant *Pseudomonas* and *Bacillus* spp. which led to increased water and nutrient uptake from rhizospheric soil (Vardharajula et al., 2011). Rhizobacterial EPS production causes improved RSA and shoot growth in plants during water deficit conditions (Ahn et al., 2007; Awad et al., 2012) thus concluding that EPS positively affects the soil aggregation and water regulation. Plant growth was promoted due to production of exopolymeric substances containing EPS by *Pseudomonas* sp. and *Acinetobacter* sp. during drought stress it was due to formation of a hydrophilic biofilm enclosing the roots that enacted as an ancillary rhizosheath securing the plant roots from soil hardness (Rolli et al., 2014). Dimitrova et al. (2013) observed that EPS through emulsification bestows security to biomembranes and in ROS scavenging thus conferring drought resistance to plants. Drought tolerance was induced in wheat plants treated by *B. thuringiensis* due to alginate production (Timmusk et al., 2014). Wheat plants inoculated with catalase and EPS producing rhizobacteria *Rhizobium leguminosarum* (LR-30), *Mesorhizobium ciceri* (CR-30 and CR-39), and *Rhizobium phaseoli* (MR-2) were able to combat drought stress; improvement in root length, biomass and drought tolerance index of the wheat seedlings were noticed that alleviated drought stress (Hussain et al., 2014). There have

been extensive studies done establishing role of raised levels of rhizobacterial EPS in alleviating drought stress conditions however more research are needed to investigate involving variations in EPS composition under drought stress.



10.7 VOLATILE PRODUCTION IN INDUCING DROUGHT TOLERANCE

VOCs are metabolites having lipophilic nature and high vapor pressure which act as signaling molecules for inter or intra kingdom communications and cell to cell signaling (Kai et al., 2009). Arabidopsis plants inoculated with *Bacillus amyloliquefaciens* IN937a and *B. subtilis* GB03 induced production of two VOCs 2R, 3R-butanediol and 3-hydroxy-2-butanone (acetoin). This modulated gene expression of genes involved in cell wall structure which promoted growth in Arabidopsis (Ryu, 2004). Root colonization of Arabidopsis with *Pseudomonas chlororaphis* O6 induced production of 2R, 3R-butanediol by *P. chlororaphis* O6 which led to stomatal closure and enhanced drought tolerance in plants; however, role of various phytohormone such as SA, ethylene, and JA was also established (Cho et al., 2008). On contrary, treatment of wheat seedlings with *B. thuringiensis* AZP2 led to increased plant biomass and survival during drought stress due to decreased emission of volatiles and increased photosynthesis (Timmusk et al., 2014). This implicated the role of rhizobacterial inoculation toward improved plant tolerance during drought (Timmusk et al., 2014).



10.8 PRODUCTION AND REGULATION OF STRESS-RESPONSIVE GENES

Gene expression analysis involving various molecular techniques is a robust tool to understand and unveil the mystic feedback responses of organisms to environmental conditions (Schlauch et al., 2010). Rhizobacterial treatment of plants leads to up regulation of genes which enhance stress tolerance. Macroarray analysis of 7200 EST (expressed sequence tags) from nodules of common bean plants treated with

Rhizobium etli revealed an up regulation of genes responsible for carbon and nitrogen metabolism as a consequence of trehalose signaling (Suarez et al., 2008). Transcriptional studies revealed rhizobacterial promoted drought tolerance in *Arabidopsis* plants inoculated with *Paenibacillus polymyxa*. With RNA differential display on parallel RNA preparations showed induction of drought responsive genes, ERD15 (Early Response to Dehydration 15) and ABA-responsive gene, RAB18 (LEA) in response to drought stress (Timmusk and Wagner, 1999). Application of BBS consortium in cucumber plants helped to sustain transcriptional levels of ribulose-1, 5-bisphosphate carboxy/oxygenase (RuBisCO) large and small subunits (rbcL and rbcS) genes essential for photosynthesis which ultimately helped plants to combat drought stress (Wang et al., 2012). Lim and Kim (2013) used two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) and differential display PCR (DD-PCR) to study changes in protein and RNA accumulation patterns in *B. licheniformis* K11 inoculated and noninoculated pepper plants. It was observed that inoculation induced expression of stress specific genes Cadhn (dehydrin-like protein), VA (Vacuolar ATPase), sHSP (Plant small heat shock proteins) and CaPR-10 (Pathogenesis-related proteins) more than a 1.5-fold in inoculated plants in comparison to control plants during drought stress. Application of RT-PCR analysis showed an upregulation of stress related genes APX1 (ascorbate peroxidase), SAMS1 (S-adenosyl-methionine synthetase), and HSP17.8 (heat shock protein) in wheat leaves inoculated with *B. amyloliquefaciens* 5113 and *A. brasilense* NO40 (Kasim et al., 2013). Illumina sequencing (HiSeq 2000 system) revealed that ABA-dependent signaling genes were activated in sugar cane cv. SP70-1143 on priming with *Gluconacetobacter diazotrophicus* PAL5 which conferred drought tolerance to plants (Vargas et al., 2014). Sarma and Saikia (2014) reported that inoculating mung plants with *Pseudomonas aeruginosa* caused an up regulation of three drought stress-responsive genes, i.e., dehydration responsive element binding protein (DREB2A), catalase (CAT1) and dehydrin (DHN) which conferred drought stress tolerance to sugarcane plants.



10.9 CONCLUSION AND FUTURE OUTLOOK

Drought stress restricts the growth of crop plants which cause significant yield losses thus causing an evident threat to food security.

Harnessing rhizobacterial plant interactions is a felicitous approach to achieve the desired food targets for the increasing population in the present scenario of climate change. Research studies have revealed that rhizobacterial inoculation in plants with PGPR modulates drought stress regulation via RIDER which are regulated by a series of signaling pathways ultimately alleviating stressed conditions. High-throughput techniques can help to unveil and understand the diligent functions of rhizobacteria in relation to various physiological and metabolic processes that play a key role in inducing RIDER in plants. In future, it will be enthralling to investigate the role of rhizobial metabolites produced under drought stress conditions. More focused research is needed to identify the receptors that lead to the expression of specific genes after the application of rhizobacteria to plants. In addition, other aspects which need to be involved in future research are (1) to study the competition between rhizobacterial strains and native soil microbes in the rhizosphere of plants grown in drought stress, (2) drought stress induced tolerance at plant tissue, cell, and molecular level.

REFERENCES

- Ahn, T.S., Ka, J.O., Lee, G.H., Song, H.G., 2007. Microcosm study for revegetation of barren land with wild plants by some plant growth-promoting rhizobacteria. *J Microbiol Biotechnol* 17, 52–57.
- Alami, Y., Achouak, W., Marol, C., Heulin, T., 2000. Rhizosphere soil aggregation and plant growth promotion of sunflowers by an exopolysaccharide-producing *Rhizobium* sp. strain isolated from sunflower roots. *Appl Environ Microbiol* 66, 3393–3398.
- Ansary, M.H., Rahmani, H.A., Ardakani, M.R., Paknejad, F., Habibi, D., Mafakheri, S., 2012. Effect of *Pseudomonas fluorescens* on proline and phytohormonal status of maize (*Zea mays* L.) under water deficit stress. *Ann Biol Res* 3, 1054–1062.
- Arkipova, T.N., Prinsen, E., Veselov, S.U., Martinenko, E.V., Melentiev, A.I., Kudoyarova, G.R., 2007. Cytokinin producing bacteria enhance plant growth in drying soil. *Plant Soil* 292, 305–315.
- Armada, E., Roldan, A., Azcon, R., 2014. Differential activity of autochthonous bacteria in controlling drought stress in native *Lavandula* and *Salvia* plants species under drought conditions in natural arid soil. *Microbial Ecology* 67, 410–420.
- Aroca, R., Ferrante, A., Vernieri, P., Chrispeels, M., 2006. Drought, abscisic acid and transpiration rate effects on the regulation of PIP aquaporin gene expression and abundance in *Phaseolus vulgaris* plants. *Ann Bot*, 98, 1301–1310.
- Arshad, M., Sharoona, B., Mahmood, T., 2008. Inoculation with *Pseudomonas* spp. containing ACC deaminase partially eliminate the effects of drought stress on growth, yield and ripening of pea (*P. sativum* L.). *Pedosphere* 18, 611–620.
- Arzanesh, M.H., Alikhani, H.A., Khavazi, K., Rahimian, H.A., Miransari, M., 2011. Wheat (*Triticum aestivum* L.) growth enhancement by *Azospirillum* sp. under drought stress. *World J Microbiol Biotechnol* 27, 197–205.

- Awad, N.M., Turkey, A.S., Abdelhamid, M.T., Attia, M., 2012. Ameliorate of environmental salt stress on the growth of *Zea mays* L. plants by exopolysaccharides producing bacteria. *J Appl Sci Res* 8, 2033–2044.
- Bangash, N., Khalid, A., Mahmood, T., Siddique, M.T., 2013. Screening rhizobacteria containing ACC-deaminase for growth promotion of wheat under water stress. *Pak J Bot* 45, 91–96.
- Bano, A., Fatima, M., 2009. Salt tolerance in *Zea mays* (L.) following inoculation with *Rhizobium* and *Pseudomonas*. *Biol Fertil Soils* 45, 405–413.
- Bano, Q., Ilyas, N., Bano, A., Zafar, N., Akram, A., Ul Hassan, F., 2013. Effect of *Azospirillum* inoculation on maize (*Zea mays* l.) under drought stress. *Pak J Bot* 45, 13–20.
- Bashan, Y., de-Bashan, L.E., Prabhu, S.R., Hernandez, J.P., 2014. Advances in plant growth-promoting bacterial inoculant technology: formulations and practical perspectives (1998–2013). *Plant Soil* 378, 1–33.
- Baxter, A., Mittler, R., Suzuki, N., 2014. ROS as key players in plant stress signaling. *J Exp Bot* 65, 1229–1240.
- Belimov, A.A., Dodd, I.C., Hontzeas, N., Theobald, J.C., Safronova, V.I., Davies, W.J., 2009. Rhizosphere bacteria containing 1-aminocyclopropane-1-carboxylate deaminase increase yield of plants grown in drying soil via both local and systemic hormone signaling. *New Phytol* 181, 413–423.
- Bharti, N., Barnawal, D., Awasthi, A., Yadav, A., Kalra, A., 2014. Plant growth promoting rhizobacteria alleviate salinity induced negative effects on growth, oil content and physiological status in *Mentha arvensis*. *Acta Physiol Plant* 36, 45–60.
- Bresson, J., Varoquaux, F., Bontpart, T., Touraine, B., Vile, D., 2013. The PGPR strain *Phyllobacterium brassicacearum* STM196 induces a reproductive delay and physiological changes that result in improved drought tolerance in *Arabidopsis*. *New Phytol* 200, 558–569.
- Cassan, F., Maiale, S., Masciarelli, O., Vidal, A., Luna, V., Ruiz, O., 2009. Cadaverine production by *Azospirillum brasilense* and its possible role in plant growth promotion and osmotic stress mitigation. *Eur J Soil Biol* 45, 12–19.
- Castillo, P., Escalante, M., Gallardo, M., Alemanno, S., Abdala, G., 2013. Effects of bacterial single inoculation and co-inoculation on growth and phytohormone production of sunflower seedlings under water stress. *Acta Physiol Plant* 35, 2299–2309.
- Chen, T.H., Murata, N., 2008. Glycine betaine, an effective protectant against abiotic stress in plants. *Trends Plant Sci* 13, 499–505.
- Cho, S.M., Kang, B.R., Han, S.H., Anderson, A.J., Park, J.Y., Lee, Y.H., et al., 2008. 2R, 3R-Butanediol, a bacterial volatile produced by *Pseudomonas chlororaphis* O6, is involved in induction of systemic tolerance to drought in *Arabidopsis thaliana*. *Mol Plant Microbe Interact* 21, 1067–1075.
- Cho, S.M., Kang, B.R., Kim, Y.C., 2013. Transcriptome analysis of induced systemic drought tolerance elicited by *Pseudomonas chlororaphis* O6 in *Arabidopsis thaliana*. *Plant Pathol J* 29, 209–220.
- Cohen, A.C., Travaglia, C.N., Bottini, R., Piccoli, P.N., 2009. Participation of abscisic acid and gibberellins produced by endophytic *Azospirillum* in the alleviation of drought effects in maize. *Botanique* 87, 455–462.
- Cohen, A.C., Bottini, R., Pontin, M., Berli, F.J., Moreno, D., Boccanlandro, H., et al., 2015. *Azospirillum brasilense* ameliorates the response of *Arabidopsis thaliana* to drought mainly via enhancement of ABA levels. *Physiol Plant* 153, 79–90.
- Creus, C.M., Graziano, M., Casanovas, E.M., Pereyra, M.A., Simontacchi, M., Puntarulo, S., et al., 2005. Nitric oxide is involved in the *Azospirillum brasilense*-induced lateral root formation in tomato. *Planta*, 221, 297–303.

- Davey, M.E., O'Toole, G.A., 2000. Molecular biofilms: from ecology to molecular genetics. *Microbiol Mol Biol Rev* 64, 847–867.
- Daviere, J.M., Achard, P., 2013. Gibberellin signaling in plants. *Development* 140, 1147–1151.
- Dimitrova, S., Pavlova, K., Lukanov, L., Korotkova, E., Petrova, E., Zagorchev, P., et al., 2013. Production of metabolites with antioxidant and emulsifying properties by Antarctic strain *Sporobolomyces salmonicolor* AL (1). *Appl Biochem Biotechnol* 169, 301–311.
- Dimkpa, C., Weinand, T., Asch, F., 2009. Plant-rhizobacteria interactions alleviate abiotic stress conditions. *Plant Cell Environ* 32, 1682–1694.
- Egamberdieva, D., Kucharova, Z., 2009. Selection for root colonizing bacteria stimulating wheat growth in saline soils. *Biol Fertil Soils* 45, 561–573.
- Fan, X., Hu, H., Huang, G., Huang, F., Li, Y., Palta, J., 2015. Soil inoculation with *Burkholderia* sp. LD-11 has positive effect on water-use efficiency in inbred lines of maize. *Plant Soil* 390, 337–349.
- Forchetti, G., Masciarelli, O., Izaguirre, M.J., Alemano, S., Alvarez, D., Abdala, G., 2010. Endophytic bacteria improve seedling growth of sunflower under water stress, produce salicylic acid, and inhibit growth of pathogenic fungi. *Curr Microbiol* 61, 485–493.
- Gill, S.S., Tuteja, N., 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol Biochem* 48, 909–930.
- Glick, B.R., 2014. Bacteria with ACC deaminase can promote plant growth and help to feed the world. *Microbiol Res*, 169, 30–39.
- Glick, B.R., Cheng, Z., Czarny, J., Duan, J., 2007. Promotion of plant growth by ACC deaminase-producing soil bacteria. In: Bakker, P.A.H., Raaijmakers, J., Lemanceau, P., Bloembergen, G. (Eds.), *New Perspectives and Approaches in Plant Growth-promoting Rhizobacteria Research*. Springer, Netherlands, pp. 329–339.
- Gou, W., Tian, L., Ruan, Z., Zheng, P., Chen, F., Zhang, L., et al., 2015. Accumulation of choline and glycine betaine and drought stress tolerance induced in maize (*Zea mays*) by three plant growth promoting rhizobacteria (pgpr) strains. *Pak J Bot*, 47, 581–586.
- Grover, M., Madhubala, R., Ali, S.Z., Yadav, S.K., Venkateswarlu, B., 2014. Influence of *Bacillus* spp. strains on seedling growth and physiological parameters of sorghum under moisture stress conditions. *J Basic Microbiol* 54, 951–961.
- Gupta, K., Dey, A., Gupta, B., 2013. Plant polyamines in abiotic stress responses. *Acta Physiol Plant* 35, 2015–2036.
- Gururani, M.A., Upadhyaya, C.P., Baskar, V., Venkatesh, J., Nookaraju, A., Park, S.W., 2013. Plant growth-promoting rhizobacteria enhance abiotic stress tolerance in *Solanum tuberosum* through inducing changes in the expression of ROS-Scavenging enzymes and improved photosynthetic performance. *J Plant Growth Regul* 32, 245–258.
- Gusain, Y.S., Singh, U.S., Sharma, A.K., 2015. Bacterial mediated amelioration of drought stress in drought tolerant and susceptible cultivars of rice (*Oryza sativa* L.). *Afr J Biotechnol* 14, 764–773.
- Hayat, S., Hayat, Q., Alyemeni, M.N., Wani, A.S., Pichtel, J., Ahmad, A., 2012. Role of proline under changing environments: a review. *Plant Signal Behav* 7, 1456–1466.
- Heidari, M., Golpayegani, A., 2011. Effects of water stress and inoculation with plant growth promoting rhizobacteria (PGPR) on antioxidant status and photosynthetic pigments in basil (*Ocimum basilicum* L.). *J Saudi Soc Agric Sci* 11, 57–61.
- Huang, Z., Zhao, L., Chen, D., Liang, M., Liu, Z., Shao, H., et al., 2013. Salt stress encourages proline accumulation by regulating proline biosynthesis and degradation in Jerusalem artichoke plantlets. *PLoS One*, 8, e62085.

- Hussain, M.B., Zahir, Z.A., Asghar, H.N., Asghar, M., 2014. Exopolysaccharides producing rhizobia ameliorate drought stress in wheat. *Int J Agric Biol* 16, 3–13.
- Iqbal, M., Ashraf, M., 2010. Changes in hormonal balance: a possible mechanism of pre-sowing chilling-induced salt tolerance in spring wheat. *J Agron Crop Sci* 196, 440–454.
- Kaci, Y., Heyraud, A., Barakat, M., Heulin, T., 2005. Isolation and identification of an EPS-producing *Rhizobium* strain from arid soil (Algeria): characterization of its EPS and the effect of inoculation on wheat rhizosphere soil structure. *Res Microbiol* 156, 522–531.
- Kai, M., Haustein, M., Molina, F., Petri, A., Scholz, B., Piechulla, B., 2009. Bacterial volatiles and their action potential. *Appl Microbiol Biotechnol* 81, 1001–1012.
- Kang, S.M., Khan, A.L., Waqas, M., You, Y.H., Kim, J.H., Kim, J.G., et al., 2014a. Plant growth-promoting rhizobacteria reduce adverse effects of salinity and osmotic stress by regulating phytohormones and antioxidants in *Cucumis sativus*. *J Plant Interact* 9, 673–682.
- Kang, S.M., Radhakrishnan, R., Khan, A.L., Kim, M.-J., Park, J.-M., Kim, B.-R., et al., 2014b. Gibberellin secreting rhizobacterium *Pseudomonas putida* H-2-3 modulates the hormonal and stress physiology of soybean to improve the plant growth under saline and drought conditions. *Plant Physiol Biochem* 84, 115–124.
- Kasim, W.A., Osman, M.E., Omar, M.N., Abd El-Daim, I.A., Bejai, S., Meijer, J., 2013. Control of drought stress in wheat using plant growth promoting bacteria. *J Plant Growth Regul* 32, 122–130.
- Kaushal, M., Wani, S.P., 2015. Plant-growth-promoting rhizobacteria: drought stress alleviators to ameliorate crop production in drylands. *Ann Microbiol* 66, 35–42.
- Lim, J.H., Kim, S.D., 2013. Induction of drought stress resistance by multi-functional PGPR *Bacillus licheniformis* K11 in Pepper. *Plant Pathol J* 29, 201–208.
- Liu, F., Xing, S., Ma, H., Du, Z., Ma, B., 2013. Cytokinin-producing, plant growth promoting rhizobacteria that confer resistance to drought stress in *Platycladus orientalis* container seedlings. *Appl Microbiol Biotechnol* 97, 9155–9164.
- Lugtenberg, B., Kamilova, F., 2009. Plant-growth-promoting rhizobacteria. *Annu Rev Microbiol* 63, 541–556.
- Mahalingam, R., 2015. Consideration of combined stress: a crucial paradigm for improving multiple stress tolerance in plants. In: Mahalingam, R. (Ed.), *Combined Stresses in Plants*. Springer-Verlag, Berlin Heidelberg, pp. 1–25.
- Marulanda, A., Barea, J.M., Azcon, R., 2009. Stimulation of plant growth and drought tolerance by native microorganisms (AM fungi and bacteria) from dry environment. Mechanisms related to bacterial effectiveness. *J Plant Growth Regul* 28, 115–124.
- Mayak, S., Tirosh, T., Glick, B.R., 2004. Plant growth promoting bacteria that confer resistance to water stress in tomato and pepper. *Plant Sci* 166, 525–530.
- Miller, G., Susuki, N., Ciftci-Yilmaz, S., Mittler, R., 2010. Reactive oxygen species homeostasis and signaling during drought and salinity stresses. *Plant Cell Environ* 33, 453–467.
- Molina-Favero, C., Creus, C.M., Simontacchi, M., Puntarulo, S., Lamattina, L., 2008. Aerobic nitric oxide production by *Azospirillum brasilense* Sp245 and its influence on root architecture in tomato. *Mol Plant Microbe Interact* 2, 1001–1009.
- Naseem, H., Bano, A., 2014. Role of plant growth-promoting rhizobacteria and their exopolysaccharide in drought tolerance of maize. *J Plant Interact* 9, 689–701.
- Naveed, M., Mitter, B., Reichenauer, T.G., Wiczeorek, K., Sessitsch, A., 2014. Increased drought stress resilience of maize through endophytic colonization by *Burkholderia phytofirmans* PsJN and *Enterobacter* sp. FD 17. *Environ Exper Bot* 97, 30–39.
- Ngumbi, E., Kloepper, J., 2014. Bacterial-mediated drought tolerance: current and future prospects. *Appl Soil Ecol* 105, 109–125.

- Nguyen, D., Rieu, I., Mariani, C., van Dam, N.M., 2016. How plants handle multiple stresses: hormonal interactions underlying responses to abiotic stress and insect herbivory. *Plant Mol Biol* 91, 727–740.
- Paul, D., Nair, S., 2008. Stress adaptations in a plant growth promoting rhizobacterium (PGPR) with increasing salinity in the coastal agricultural soils. *J Basic Microbiol* 48, 378–384.
- Pereyra, M.A., Garcia, P., Colabelli, M.N., Barassi, C.A., Creus, C.M., 2012. A better water status in wheat seedlings induced by *Azospirillum* under osmotic stress is related to morphological changes in xylem vessels of the coleoptile. *Appl Soil Ecol* 53, 94–97.
- Postma, J.A., Lynch, J.P., 2011. Root cortical aerenchyma enhances growth of *Zea mays* L. on soils with suboptimal availability of nitrogen, phosphorus and potassium. *Plant Physiol* 156, 1190–1201.
- Raza, A., Faisal, M., 2013. Growth promotion of maize by desiccation tolerant *Micrococcus luteus*-chp37 isolated from Cholistan desert, Pakistan. *Aust J Crop Sci* 7, 1693–1698.
- Reina-Bueno, M., Argandona, M., Nieto, J.J., Hidalgo-Garcia, A., Iglesias-Guerra, F., Delgado, M.J., et al., 2012. Role of trehalose in heat and desiccation tolerance in the soil bacterium *Rhizobium ethi*. *BMC Microbiol* 12, 207.
- Rodriguez, S.J., Suarez, R., Caballero, M.J., Itturiaga, G., 2009. Trehalose accumulation in *Azospirillum brasilense* improves drought tolerance and biomass in maize plants. *FEMS Microbiol Lett* 296, 52–59.
- Rolli, E., Marasco, R., Vigani, G., Ettoumi, B., Mapelli, F., Deangelis, M.L., et al., 2014. Improved plant resistance to drought is promoted by the root-associated microbiome as a water stress-dependent trait. *Environ Microbiol* 17, 316–331.
- Rossi, F., Potrafka, R.M., Pichel, F.G., De Philippis, R., 2012. The role of the exopolysaccharides in enhancing hydraulic conductivity of biological soil crusts. *Soil Biol Biochem* 46, 33–40.
- Ryu, C.M., 2004. Bacterial volatiles induce systemic resistance in *Arabidopsis*. *Plant Physiol* 134, 1017–1026.
- Salomon, M.V., Bottini, R., de Souza Filho, G.A., Cohen, A.C., Moreno, D., Gil, M., et al., 2014. Bacteria isolated from roots and rhizosphere of *Vitis vinifera* retard water losses, induce abscisic acid accumulation and synthesis of defense-related terpenes in in vitro cultured grapevine. *Physiol Plant* 51, 359–374.
- Sang-Mo, K., Radhakrishnan, R., Khan, A.L., Min-Ji, K., Jae-Man, P., Bo-Ra, K., Dong-Hyun, S., In-Jung, L., 2014. Gibberellin secreting rhizobacterium, *Pseudomonas putida* H-2-3 modulates the hormonal and stress physiology of soybean to improve the plant growth under saline and drought conditions. *Plant Physiol. Biochem.* 84, 115–124.
- Sarma, R.K., Saikia, R., 2014. Alleviation of drought stress in mung bean by strain *Pseudomonas aeruginosa* GGRJ21. *Plant Soil* 377, 111–126.
- Schlauch, K.A., Grimplet, J., Cushman, J., Grant, R.C., 2010. Transcriptomics analysis methods: microarray data processing, analysis and visualization using the affymetrix gene chip® *Vitis Vinifera* genome array. In: Delrot, S., Medrano, H., Bavaresco, L., Grando, S. (Eds.), *Methodologies and Results in Grapevine Research*. Springer, Dordrecht, pp. 317–334.
- Selvakumar, G., Panneerselvam, P., Ganeshamurthy, A.N., 2012. Bacterial mediated alleviation of abiotic stress in crops. In: Maheshwari, D.K. (Ed.), *Bacteria in Agrobiolgy: Stress Management*. Springer-Verlag, Berlin Heidelberg, pp. 205–224.
- Shintu, P.V., Jayaram, K.M., 2015. Phosphate solubilising bacteria (*Bacillus polymyxa*)—an effective approach to mitigate drought in tomato (*Lycopersicon esculentum* Mill). *Trop Plant Res* 2, 17–22.

- Simontacchi, M., Galatro, A., Ramos-Artuso, F., Santa-Maria, G.E., 2015. Plant survival in a changing environment: the role of nitric oxide in plant responses to abiotic stress. *Front Plant Sci* 6, 977.
- Suarez, R., Wong, A., Ramirez, M., Barraza, A., OrozcoMdel, C., Cevallos, M.A., et al., 2008. Improvement of drought tolerance and grain yield in common bean by over expressing trehalose-6-phosphate synthase in rhizobia. *Mol Plant Microbe Interact* 21, 958–966.
- Timmusk, S., Wagner, E.G., 1999. The plant-growth-promoting rhizobacterium *Paenibacillus polymyxa* induces changes in *Arabidopsis thaliana* gene expression: a possible connection between biotic and abiotic stress responses. *Mol Plant Microbe Interact* 12, 951–959.
- Timmusk, S., Paalme, V., Pavlicek, T., Bergquist, J., Vangala, A., Danilas, T., et al., 2011. Bacterial distribution in the rhizosphere of wild barley under contrasting microclimates. *PLoS One* 6, e17968.
- Timmusk, S., Abd El-Daim, I.A., Copolovici, L., Tanilas, T., Kannaste, A., Behers, L., et al., 2014. Drought-tolerance of wheat improved by rhizosphere bacteria from harsh environments: enhanced biomass production and reduced emissions of stress volatiles. *PLoS One* 9, e96086.
- Vacheron, J., Desbrosses, G., Bouffaud, M.L., Touraine, B., Moenne-Loccoz, Y., Muller, D., et al., 2013. Plant growth-promoting rhizobacteria and root system functioning. *Front Plant Sci* 4, 356.
- Vardharajula, S., Ali, S.Z., Grover, M., Reddy, G., Venkateswaralu, B., 2010. Effect of plant growth promoting *Pseudomonas* spp. on compatible solutes antioxidant status and plant growth of maize under drought stress. *Plant Growth Regul* 62, 21–30.
- Vardharajula, S., Ali, S.Z., Grover, M., Reddy, G., Venkateswarlu, B., 2011. Drought-tolerant plant growth promoting *Bacillus* spp. effect on growth, osmolytes, and antioxidant status of maize under drought stress. *J Plant Interact* 6, 1–14.
- Vargas, L., Santa Brigida, A.B., Mota Filho, J.P., de Carvalho, T.G., Rojas, C.A., Vanechoutte, D., et al., 2014. Drought tolerance conferred to sugarcane by association with *Gluconacetobacter diazotrophicus*: a transcriptomic view of hormone pathways. *PLoS One* 9, e114744.
- Vu, B., Chen, M., Crawford, R.J., Ivanova, E.P., 2009. Bacterial extracellular polysaccharides involved in biofilm formation. *Molecules* 14, 25–35.
- Vurukonda, S.S.K.P., Vardharajula, S., Shrivastava, M., Ali, S.K.Z., 2016. Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria. *Microbiol Res* 184, 13–24.
- Wang, C.J., Yang, W., Wang, C., Gu, C., Niu, D.D., Liu, H.X., et al., 2012. Induction of drought tolerance in cucumber plants by a consortium of three plant growth-promoting rhizobacterium strains. *PLoS One* 7, e25265.
- Wang, Y., Ohara, Y., Nakayashiki, H., Tosa, Y., Mayama, S., 2005. Microarray analysis of the gene expression profile induced by the endophytic plant growth promoting rhizobacteria, *Pseudomonas fluorescens* FPT9601-T5 in *Arabidopsis*. *Mol Plant Microbe Interact* 18, 385–396.
- Weiner, J.J., Peterson, F.C., Volkman, B.F., Cutler, S.R., 2010. Structural and functional insights into core ABA signaling. *Curr Opin Plant Biol* 5, 495–502.
- Xu, J., Li, X.L., Luo, L., 2012. Effects of engineered *Sinorhizobium meliloti* on cytokinin synthesis and tolerance of alfalfa to extreme drought stress. *Appl Environ Microbiol* 78, 8056–8061.
- Yang, S., Vanderbeld, B., Wan, J., Huang, Y., 2010. Narrowing down the targets, towards successful genetic engineering of drought-tolerant crops. *Mol Plant*, 3, 469–490.

- Zahir, Z.A., Munir, A., Asghar, H.N., Arshad, M., Shaharoona, B., 2008. Effectiveness of rhizobacteria containing ACC-deaminase for growth promotion of peas (*Pisum sativum*) under drought conditions. *J Microbiol Biotechnol* 18, 958–963.
- Zhang, H., Murzello, C., Sun, Y., Kim, Mi-S., Jeter, R.M., Zak, J.C., et al., 2010. Choline and osmotic-stress tolerance induced in *Arabidopsis* by the soil microbe *Bacillus subtilis* (GB03). *Mol Plant Microbe Interact* 23, 1097–1104.
- Zhou, C., Ma, Z., Zhu, L., Xiao, X., Xie, Y., Zhu, J., et al., 2016. Rhizobacterial strain *Bacillus megaterium* BOFC15 induces cellular polyamine changes that improve plant growth and drought resistance. *Int J Mol Sci*, 17 (6), 976.
- Zhou, S., Hu, W., Deng, X., Ma, Z., Chen, L., Huang, C., et al., 2012. Overexpression of wheat aquaporin gene, TaAQP7 enhances drought tolerance in transgenic tobacco. *PLoS One* 7, e52439.



Isolation and Characterization of Plant Growth Promoting Rhizobacteria From *Momordica Charantia* L.

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11.1 INTRODUCTION

Momordica charantia L. is most common vegetable crop, belongs to family Cucurbitaceae, and commonly known as bitter melon, bitter gourd, and Karela in India. It is widely cultivated and utilized as nutritious as well as medicinal plant in the households of Indian sub-continent. Bitter gourd is the leading vegetable crop and the high yields and maximum economic return made them most preferred vegetable crop in India. It contains various metabolites and nutritional compounds that help in suppressing a range of diseases and act as antidiabetic, anticancerous, antimicrobial, antioxidant antihelminthic, antitumorous, antimutagenic, antiHIV, antifertility, abortifacient, and antimicrobial source (Bakare et al., 2010; Singh et al., 2012; Joseph and Jini, 2013; Ozusaglam and Karakoca, 2013).

Rhizosphere of the plants is the most prominent zone for microbial interaction due to the chemical exudates secreted by the roots. Root exudates contain polysaccharide, lipids, and amino acids, which directly involved in microbial interaction with plant (Kumar et al., 2015b). The rhizosphere of *M. charantia* harbors diverse groups of microorganism such as bacteria, actinomycetes, fungi, and nematode. Some of them harmful for the plants whereas large number of the strains directly or indirectly involved in growth promotion and disease management in plants (Glick, 2012; Kumar et al., 2014, 2015b, 2016a). The direct promotion by PGPR entails the plant growth promoting substance synthesized or

facilitating the uptake of certain nutrients (Ahmad et al., 2006). These microbes benefit the plant growth directly through various mechanisms such as phosphate solubilization, siderophore production, NH_3 production, and also through the synthesis of phytohormones (Glick, 2014; Kumar et al., 2016a,b). The indirect mechanism of plant growth occurs through, when PGPR antagonize the deleterious effect of one or more phytopathogenic micro-organisms. In the last few decades, a large array of bacteria including species of *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthobacter*, *Burkholderia*, *Bacillus*, and *Serratia* have been reported to enhance plant growth (Kloepper et al., 1980; Okon and Labandera-Gonzalez, 1994). *Pseudomonas* and *Bacillus* are the most commonly investigated PGPR, and often dominate the rhizosphere (Morgan et al., 2005; Kumar et al., 2016b).

Currently agriculture largely depends upon the chemical fertilizers, growth regulators, and pesticides to attain maximum yields. Continuous use of chemical adversely affect the environment, nutrient recycling and causes health hazards, interruption of ecology, destruction of microbial communities. In this context, PGPR considered as one of the novel and potential tool to provide substantial benefits to agriculture (Kumar et al., 2013). The limited number of studies is available on the bacterial diversity in the rhizosphere, primarily due to lack of appropriate techniques to isolate sufficient number of strains of the same species. The diversity of microflora in the rhizosphere depends upon organic exudates, which may eventually vary with the stage of plant growth (Wieland et al., 2001).

The present investigation deals with isolation of rhizospheric strains of *M. charantia* and their plant growth promotion trait analysis, stress tolerance, antibiotic activity and also their C and N utilization pattern. Which enhance the understanding of rhizospheric diversity of *M.charantia* and their utilization in sustainable agriculture.



11.2 MATERIALS AND METHODS

11.2.1 Study site, sampling, and bacterial isolation

Momordica charantia L. (Bitter gourd) was grown in earthen pot with natural soil amended with farmyard manure and sand (2:1:1) in the Botanical garden of Banaras Hindu University campus (25°20' N and 83°00' E,

elevation 80.71 m). Rhizospheric soil of *M. charantia* were collected at the flowering stage (35–40 days) of the plant.

Rhizospheric soil (fresh) suspended in 50 ml buffer (phosphate 10 mM, pH-7.0) was shaken vigorously (1 h) on a gravatory shaker and serially diluted up to 10^{-5} . 0.5 ml of (10^{-5}) dilution were inoculated on the Luria nutrient agar media (10 g NaCl, 10 g tryptone, 5 g yeast extract, 15 g agar, and 1 L distilled water) plate (Kumar et al., 2015b, 2016b). The plates were incubated at 30°C for 2–7 days. The bacterial colonies were selected on the basis of colony morphology and visual growth. Clones were picked up and purified by restreaking on nutrient agar. Pure clones were restreaked on agar slants for maintenance.

11.2.2 Morphological and biochemical characterization of isolates

Colony morphology (shape, size, elevation, surface, margin, color, optical characteristics, and pigmentation) of the isolated species were examined using Luria nutrient agar media following 3–5 days of incubation. Motility of bacteria was observed by hanging drop method. Bacterium in nigrosin under a cover slip hanged on a hollow slide and observed under microscope for motility (Reference). The identification and characterization of bacterial isolates were performed on the basis of colony morphology and biochemical screening according to Bergey's Manual of Determinative Bacteriology, ninth ed. (1994).

11.2.3 Identification of bacterial isolates by 16S r RNA amplification

11.2.3.1 16S rRNA gene amplification and sequencing

Genomic DNA was isolated using GeneiPure™ bacterial DNA purification kit (GeNei™, Bangaluru, India) according to the manufacture's protocol. Universal eubacterial primers F-D1-5'-ccgaattcgtcgacaacagagtttgatcctggctcag-3' and R-D1- 5'-cccgggatccaagcttaaggaggtgatccagcc-3' (Kumar et al., 2015a, 2016a, b), were used to amplify the 1500 bp region of 16S rRNA gene using a thermal cycler (BioRad USA). Amplified products were resolved by electrophoresis in agarose (1%), and visualized in the gel documentation system (Alfa Imager, Alfa InfoTech Corporation, USA). The amplicons were purified using GeneiPure™ quick PCR purification kit (GeNei™, Bangaluru, India) and quantified at 260 nm taking calf thymus DNA as control. The purified partial 16S r DNA amplicon

was sequenced in Applied Biosystems 3130 Genetic Analyzer (Applied Biosystems[®], CA, and USA).

11.2.3.2 PCR Condition

The 16S rRNA gene was amplified with universal primers 27Fs'/16F (AGAGTTTGATCMTGGCTCAG) and 1492Rs'/16R (GGYTACCTT GTTACGACTT). The reaction mixture was incubated in a Thermal Cycler with the following cycling conditions: denaturation at 94°C (3 min) and 35 cycles (94°C) for 60s, 50°C for 60s, 72°C for 2 min. followed by one cycle of elongation (10 min) at 72°C and the last for 1 h (4°C). A negative control of sterile deionized water and a positive control (*E. coli* genomic DNA) were included in the assay to confirm the validity.

11.2.3.3 Agarose gel electrophoresis

To confirm the targeted PCR amplifications, 10 µl of the PCR product from each tube was mixed with 2 µl of 6X DNA loading dye and electrophoresed along with 1.6 kb Λ marker on 0.7% agarose gel containing 1 µl of the 10 mg/ml solution of ethidium bromide at 100 V (90 min) in 0.5X TBE buffer. Λ marker was used to estimate the size of the amplified fragments. The amplified DNA product was visualized as single compact band of the expected size under UV in a gel documentation system.

11.2.3.4 Gel elution

Single compact band of 1.6 kb cut with Nucleospin kit, in the solubilizing solution was placed at 55°C, vortexed to dissolve the gel. After several loadings and washings with buffer, the solution was stored at -20°C.

11.2.3.5 Quantification of DNA

The DNA quantity was estimated with the Nanodrop 0.5 µL–2.0 µL (Thermo Scientific, USA) spectrophotometer and spectrofluorimetric analysis for the quantification of the DNA.

11.2.3.6 Analysis of 16S r DNA sequences

The 16S rRNA gene partial sequences of the isolated strains were sequenced and compared with the RNA databases. The resulting nucleotide sequence were assigned for bacterial taxonomic affiliation based on the closest match to the sequences available at National Center for Biotechnology Information (NCBI) BLAST server (www.ncbi.nlm.nih.gov).

gov/BLAST) using Nucleotide Basic Local Alignment Search Tool (BLAST) program. The sequences showing >98% similarity were retrieved. The cluster analysis of the sequence was done using the multiple sequences alignment tool, Clustal X2.1 version. The phylogenetic and molecular analyses were conducted using MEGA 5.1 (Kumar et al., 2015a, 2016a, b).

11.2.4 Plant growth promoting traits of bacterial isolates

The bacterial isolates were screened for their plant growth promoting properties including indole acetic acid production (IAA) (Brick et al., 1991), phosphate solubilization (Laslo et al., 2012), HCN production (Lorck, 1948), siderophore production (Schwyn and Neilands, 1987), and NH₃ production (Cappuccino and Sherman, 1992), as per the standard protocols.

11.2.5 Antibiotic sensitivity test

Sensitivity test was performed using antibiotic impregnated discs (6 mm dia.). Antibiotic sensitivity of the strains was tested against chloramphenicol, spectinomycin, erythromycin, rifampicin, and polymixin B by Kirby Bauer disc-diffusion method (Kumar et al., 2015a, 2016a, b). Based on the inhibition zones, organisms were categorized as resistant or sensitive according to DIFCO Manual, 10th edition (1984).

11.2.6 Carbon and Nitrogen utilization

Carbohydrate utilization was tested by modifying the Simmon's Citrate medium with the mixture of amino acids (i.e., glutamine, cysteine, methionine, alanine, and tryptophan in equal amount), A₆ trace element and multi-vitamin. Sodium citrate was replaced with 0.2% (w/v) of different carbohydrates in growth medium. Growth was examined after 2 days incubation and compared with the negative control. Nitrogen utilization was tested by adding 0.1% (w/v) different nitrogen sources in the glucose medium. Growth was examined after incubation of 2 days (Kumar et al., 2016a).

11.2.7 Stress tolerance

The selected bacterial strains were tested for their tolerance to salt stress by exposing the cells to various NaCl concentrations (1%–12%) and NaN₃ (0.02%). The culture tubes were incubated at 30°C for (48 h) and absorbance recorded at 600 nm (UV/VIS Spectrophotometer 117, Systronics, India) (Shukla, 2009; Kumar et al., 2016a, b).

11.2.8 Lysis in SDS

Lysis of bacterial cells in sodium dodecyl sulfate (SDS) was determined in microcuvettes containing 1 ml culture and SDS (0.2 and 2%). The reaction mixture was vortexed and absorbance measured at 600 nm (UV/VIS Spectrophotometer 117, Systronics, India).

Statistical analysis - The data were analyzed by using compare mean, Multiple Tukey HSD, SPSS.16, and all the data were significant up to $p < 0.05$.



11.3 RESULTS

A total of 42 rhizobacterial clones had been isolated from the rhizosphere soils of *Momordica charantia*, which were differentiated into 28 isolates on the basis of colony morphology. Twelve strains out of 28 strains had been differentiated on the basis of culture, morphology, and biochemical tests.

Details variations existed in the cultural characteristics and morphological properties (based on 3–4 days old colonies) were described in [Table 11.1](#). The colony size of four isolated strains were of diameter less than 0.5 mm, while six strains had larger than 2 mm. The remaining strains had colony diameter in the range of 0.5 mm–2 mm. Colonies of the strains were white, creamy, or light yellow.

11.3.1 Morphological and biochemical characteristics

All the strains were bacilli except strain RS8 (coccus). Most of the strains were Gram positive except strains R7, R12, RS11, and RS12 (Gram negative) in nature ([Fig. 11.1](#)). Except strains RS1 and RS5, all the strains were motile in nature. During catalase active test, all the strains showed positive response, where as only one-third of the strains were oxidase positive. Among 12 strains, three-fourth fermented carbohydrate during biochemical activity test, 5 strains were urease +ve while 2 strains produced H_2S . Most of the strains utilized citrate (except strain RS4, RS11) and positive for indole test except RS7. Most of the strains hydrolyze starch except RS13 and reduced nitrate (except R12). Details of the biochemical characteristics of the isolated strains described in [Table 11.2](#).

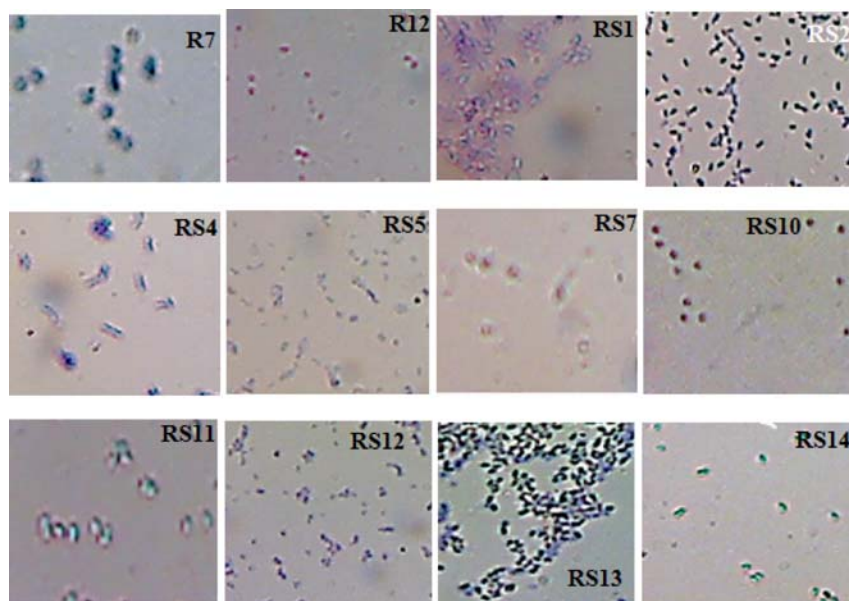


Figure 11.1 Microscopic observation of isolated strains of *Momordica charantia*.

11.3.2 Phylogenetic analysis

The species level confirmation of isolated rhizospheric strains had been performed on the basis of 16S r RNA gene sequence analysis and their sequence has been deposited in NCBI for accession number of the strains. The sequence analysis revealed the species level of strains and confirmed as (*B. amyloliquefaciens* RS2 (Accession no. JX472919), *B. mucilaginous* RS4 (Accession no. JX472921), *B. subtilis* RS5 (Accession no. JX472922), *B. subtilis* R7 (Accession no. JX472916), *Pseudomonas sp* RS10 (Accession no. JX472926), *A. calcoaceticus* R12 (Accession no. JX472918), *B. aerius* RS12 (Accession no. JX472927), *Bacillus sp.* RS13 (Accession no. JX472928), *Bacillus amyloliquefaciens* RS14 (Accession no. JX472729) on the basis of BLAST analysis. Remaining strains *Stenotrophomonas sp* RS1, *Azotobacter sp* RS7 and *Ralstonia* RS11 were identified on the basis of morphology and biochemical characteristics. The observed phylogenetic breadth covered β - and γ - Proteobacteria and firmicutes were dominant domin and *Bacillus* is the dominant group in the rhizosphere of *M. charantia*. The details of the strains and the nearest relative based on 16S rRNA gene sequence are given in [Table 11.3](#). The DNA sequence of the isolated trains were aligned and phylogenetic tree constructed by neighbor-joining method using MEGA 5.01 ([Fig. 11.2](#))

Table 11.2 Phenotypic, biochemicals and plant growth promoting traits characteristics of rhizospheric isolates of *M. charantia*

Characteristics	Cell shape	Gram reaction	Pigment	Motility	Oxidase	Citrate	Indole	H ₂ S production	Urease	Starch hydrolysis	Nitrate reduction	Catalase	Phosphate solubilizatio	IAA production	Siderophore productionn	Ammonia production	HCN production
R7	R	+	—	+	+	+	—	—	—	+	+	+	+	+	+	+	+
R12	R	—	—	—	—	+	—	—	—	+	—	+	+	+	+	+	—
RS1	C	+	—	+	—	+	—	+	+	+	+	+	+	+	+	+	+
RS2	R	+	—	+	—	+	—	—	—	+	+	+	+	+	+	+	—
RS4	R	+	—	+	—	—	—	—	+	+	+	—	+	+	+	+	—
RS5	R	+	—	—	—	+	—	—	—	+	+	+	+	+	+	+	+
RS7	R	—	+	+	+	+	+	—	+	+	+	+	+	+	—	+	—
RS10	R	+	+	+	+	+	—	+	+	+	+	+	+	+	+	+	+
RS11	R	—	—	+	—	—	—	—	—	+	+	—	+	+	—	+	—
RS12	R	—	—	+	—	+	—	—	+	+	+	+	+	+	+	+	—
RS13	R	+	—	+	—	+	—	—	—	—	+	—	+	+	—	+	+
RS14	R	+	—	+	+	+	—	—	—	+	+	+	+	+	—	+	—

Table 11.3 Closest relative of the isolated strains as revealed by 16S rRNA gene sequencing

S.N	Strains	Accession number	Nearest phylogenetic neighbor	Phylogenetic domain
1	R7	JX472916	Bacillus subtilis strain Bs16, 16S ribosomal RNA, partial sequence	Firmicutes
2	R12	JX472918	Acinetobacter calcoaceticus strain DC2 16S ribosomal RNA gene, partial sequence	γ - proteobacteria
3	RS1		Stenotrophomonas maltophilia strain KJ16S ribosomal RNA gene, partial sequence	β - proteobacteria
4	RS2	JX472919	Bacillus amyloliquefaciens subsp. plantarum gene for 16S rRNA, partial sequence, strain: M20J	Firmicutes
5	RS4	JX472921	Bacillus mucilaginous strain BMH 16S ribosomal RNA gene, partial sequence	Firmicutes
6	RS5	JX472922	Bacillus subtilis strain BVC21 16S ribosomal RNA gene, partial sequence	Firmicutes
7	RS7		Azotobacter sp	γ - proteobacteria
8	RS10	JX472926	Uncultured Pseudomonas sp. clone ASC694 16S ribosomal RNA gene, partial sequence	γ - proteobacteria
9	RS11		Ralstonia sp. HDL 16S ribosomal RNA gene, partial sequence	β - proteobacteria
10	RS12	JX472927	Bacillus aerius strain:24 K, 16S ribosomal RNA, partial sequence	Firmicutes
11	RS13	JX472928	Bacillus sp. KV1 16S ribosomal RNA gene, partial sequence	Firmicutes
12	RS14	JX472729	Bacillus amyloliquefaciens strain APS3 3 16S ribosomal RNA gene, partial sequence	Firmicutes

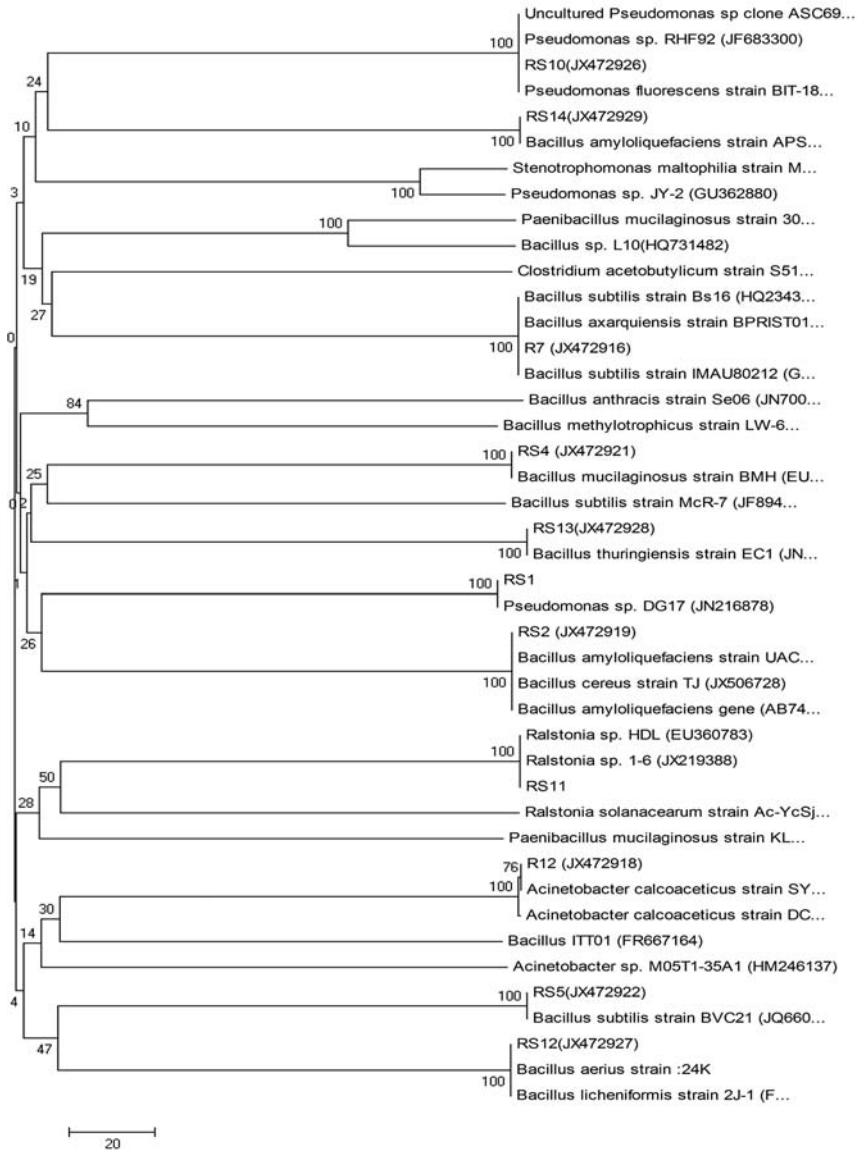


Figure 11.2 Phylogenetic tree from analysis of 16S rRNA gene sequence of the rhizospheric strains of *M. charantia* using neighbor joining approach. Each number on a branch indicates the bootstrap confidence values correspond to the scale bar of branch lengths. GenBank accession numbers of nucleotide sequences are shown along with the name of bacterial strain. Phylogenetic analyses were conducted in MEGA 5.

11.3.3 Plant growth promoting analysis

All the strains produced IAA and ammonia during PGP traits analysis, where as 75% strains solubilized tri-calcium phosphate during phosphate solubilization except RS4, RS11, and RS13 strains. During siderophore production test, most of the strains were positive during the test except the strains RS7, RS11, RS13, and RS14, where as only strains R7, RS1, RS5, RS10, and RS13 produced HCN during analysis (Table 11.2).

11.3.4 Carbon and Nitrogen source utilization

Carbon and nitrogen source utilization pattern of the rhizobacterial isolates presented in Table 11.4. The data revealed that all the PGPR strains invariably utilized glucose, sucrose, and yeast extract as the C source.

Table 11.4 Carbon and nitrogen source utilization patterns of PGPR bacteria isolated from root or *Momordica charantia*

C source	R7	R12	RS1	RS2	RS4	RS5	RS7	RS10	RS11	RS12	RS13	RS14
Glucose	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+
Trisodium citrate	+	+	+	+	—	—	+	—	+	+	+	+
Sodium acetate	—	—	+	+	—	—	+	—	—	—	—	+
Sodium formate	—	—	+	+	—	—	+	—	—	—	—	+
Mannitol	—	—	+	+	—	—	+	—	—	—	—	+
Malic acid	—	—	—	—	—	—	—	—	—	—	—	—
Methanol	—	—	—	+	—	—	—	—	—	—	+	+
Yeast extract	+	+	+	+	+	+	+	+	+	+	+	+
N source												
Potassium nitrate	—	+	+	—	+	—	+	+	+	+	+	+
Sodium nitrite	—	—	—	+	+	—	+	+	+	—	—	+
Amm. acetate	+	+	—	+	+	+	+	+	—	—	—	+
Amm. sulfate	+	+	+	+	+	+	+	+	+	+	+	+
Amm. chloride	+	+	—	+	+	+	+	+	+	+	+	+
Alanine	+	+	—	+	+	+	+	+	+	+	+	+
Lysine	+	+	+	—	+	+	+	+	+	+	+	—
Glycine	+	+	+	—	+	+	+	+	+	+	+	—

(Continued)

Table 11.4 (Continued)

C source	R7	R12	RS1	RS2	RS4	RS5	RS7	RS10	RS11	RS12	RS13	RS14
Glutamine	+	+	+	+	+	+	+	+	+	+	+	+
Isoleucine	—	+	+	—	—	—	+	+	+	—	—	—
Arginine	—	+	—	+	+	—	+	+	+	+	+	+
Cysteine	—	—	—	+	+	—	+	+	+	+	+	+
Aspartic acid	—	—	—	—	—	—	—	+	+	+	—	—
Glutamic acid	—	—	+	—	+	—	+	+	+	—	—	—
Proline	+	+	+	+	+	+	+	+	+	+	+	+

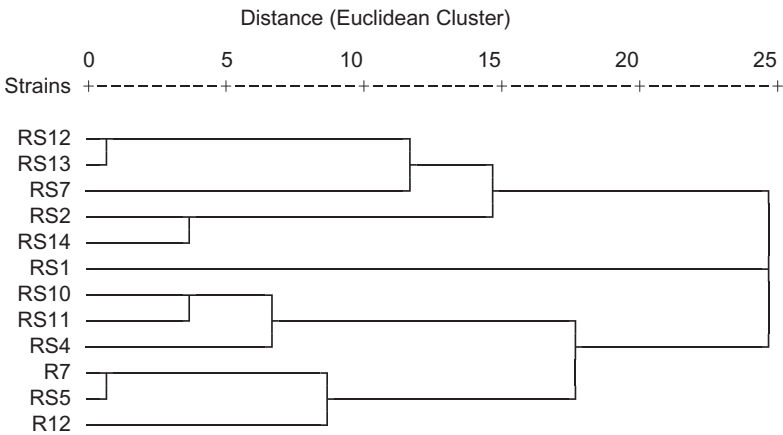


Figure 11.3 Hierarchical cluster analysis (between groups linkage) measuring the squared Euclidean distance based on their C and N utilization pattern.

Sodium acetate, formate, mannitol, and citrate were utilized by 75% of the strains while one-third of the strains utilized methanol. None of the strains utilized malic acid as the C source. The variation among the C utilization pattern was more pronounced than the N utilization pattern. All the isolates utilized ammonium sulfate, glutamine, and proline. Sodium nitrite, isoleucine, aspartic acid, and glutamic acid were utilized by a limited numbers of bacterial strains. Majority of the isolates utilized potassium nitrate, ammonium acetate, arginine, glycine, lysine, and cystiene.

Cluster analysis of strains based on their C and N utilization pattern, revealed that strains showing affinity to the same genera were placed nearby (Fig. 11.3). Strain R7 and RS5 showed resemblance to *Bacillus subtilis* while RS2 and RS14 resembled *Bacillus amyloliquifaciens* placed nearby.

11.3.5 Antibiotic sensitivity

Inhibition zone by different antibiotics indicated that Chloremphenicol (except R12), Spectinomycin (except RS11) and Kannamycin (except RS10, RS11) were the most effective antibiotics against all the isolated strains, while Polymixin B (except RS1, RS2, and RS14) was the least effective as diameter wise antibiotic. Erythromycin and Rifampicin was the mild effective antibiotic against the rhizospheric strains of *M.charentia* (Table 11.5, Fig. 11.4).

11.3.6 Stress tolerance

All the strains tolerated 4% of NaCl but their response differed at high concentration of NaCl. Strains R7, RS2, RS4, RS7, RS12, and RS14 shown maximum salinity tolerance up to 10% of NaCl. Under the stress condition of 0.02% sodium azide only five strains (R7, R12, RS1, RS5, and RS14) exhibited growth (Table 11.5).



11.4 DISCUSSIONS

Environment friendly sustainable agriculture is preferred to obtain the desirable yields in current scenario of global warming conditions. In this context, PGPR are applied in a wide variety of agro and allied industries as inoculants, for the maintenance of soil fertility and achieving desirable yields of plant (Fig. 11.5) (Aarons et al., 2000; Kumar et al., 2016b). PGPR, exert beneficial effect on the plant growth through the production of phytohormones, HCN, ammonia, siderophores, antimicrobial agents, phosphate solubilization, mineral uptake, and suppression of deleterious organisms (Maier et al., 2009; Glick, 2014; Kumar et al., 2014). There are several reports on the advantages of PGPR from vegetable plants particularly Turmeric, Mangoginger, Garlic, and Tomato (Kumar et al., 2016a,b; Jasim et al., 2014) however, studies of bitter melon, a vegetable with nutritional and pharmaceutical value and their PGPR and impact of PGPR on growth and yield of plants are almost lacking.

The bacterial community structure in the rhizosphere, depend upon root exudates and the growth stages of plants (Oku et al., 2012). The rhizospheric isolates of the *M. charentia* were identified on the basis of

Table 11.5 Antibiotic sensitivity and stress tolerance test rhizospheric strains of *Momordica charantia* (+ = positive,- = negative)

Strain	Antibiotic sensitivity (antibiotics inhibition zone in mm)						Salinity tolerance (NaCl)					NaN3
	Chloramphenicol (30 mcg/disc)	Polymixin-B (300 unit/disc)	Erythromycin (15 mcg/disc)	Rifampicin (5 mcg/disc)	Spectinomycin (30 mcg/disc)	Kanamycin (10 mcg/disc)	4%	6%	8%	10%	0.02%	
R7	24	15	—	23	46	44	+	+	+	+	+	+
R12	—	16	—	—	16	35	+	+	+	—	+	+
RS1	50	—	28	—	50	20	+	—	—	—	+	+
RS2	21	—	10	18	44	21	+	+	+	+	—	—
RS4	42	10	27	12	29	23	+	+	+	+	—	—
RS5	14	18	—	20	41	39	+	+	—	—	+	+
RS7	29	12	15	13	33	21	+	+	+	+	—	—
RS10	12	10	—	14	24	—	+	+	—	—	—	—
RS11	44	24	38	—	—	—	+	—	—	—	—	—
RS12	45	14	31	29	21	36	+	+	+	+	—	—
RS13	42	10	27	21	35	41	+	+	+	—	—	—
RS14	23	—	11	36	46	26	+	+	+	+	+	+

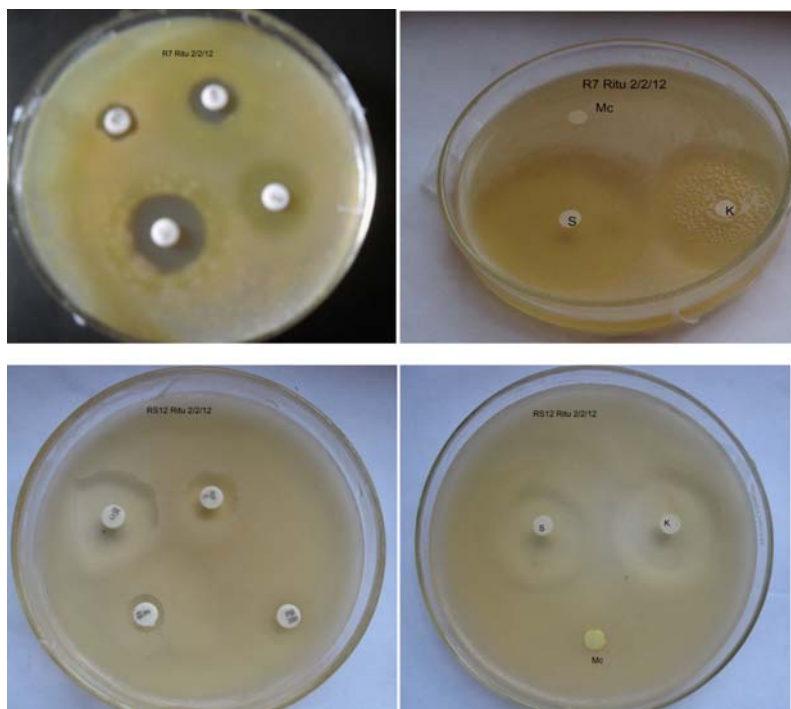


Figure 11.4 Antibiotic sensitivity of PGPR strains R7 and RS12.

morphology, biochemical, and molecular 16S rRNA gene sequence analysis. The PGPR isolates showed close similarity with genera *Bacillus*, *Acinetobacter*, and *Uncultured Pseudomonas* on the basis of 16S rRNA sequencing, whereas *Azotobacter*, *Pseudomonas*, and *Ralstonia* were identified based on morphological and biochemical analysis. *Bacillus* were the dominant species in the rhizosphere of bitter melon. *Bacillus* are ubiquitous and frequently reported from various plants as the PGPR strain (Jasim et al., 2014; Kumar et al., 2016b). *Ralstonia*, *Bacillus*, *Stenotrophomonas*, and *Pseudomonas* sp. also reported as isolates from the soil (Garbeva et al., 2003).

Phosphorous (P) is one of the most essential minerals elements that limits the productivity of plant and soil. Phosphate (H_2PO_4^-) is the utilizable form by the plants from soil solution but this form is limited even the phosphorous is abundantly present in the soil. Phosphate solubilizing bacteria frequently used in solubilizing insoluble form of phosphate to soluble form, which make easily available for the plant species. Microorganisms involved in solubilization of insoluble phosphorus

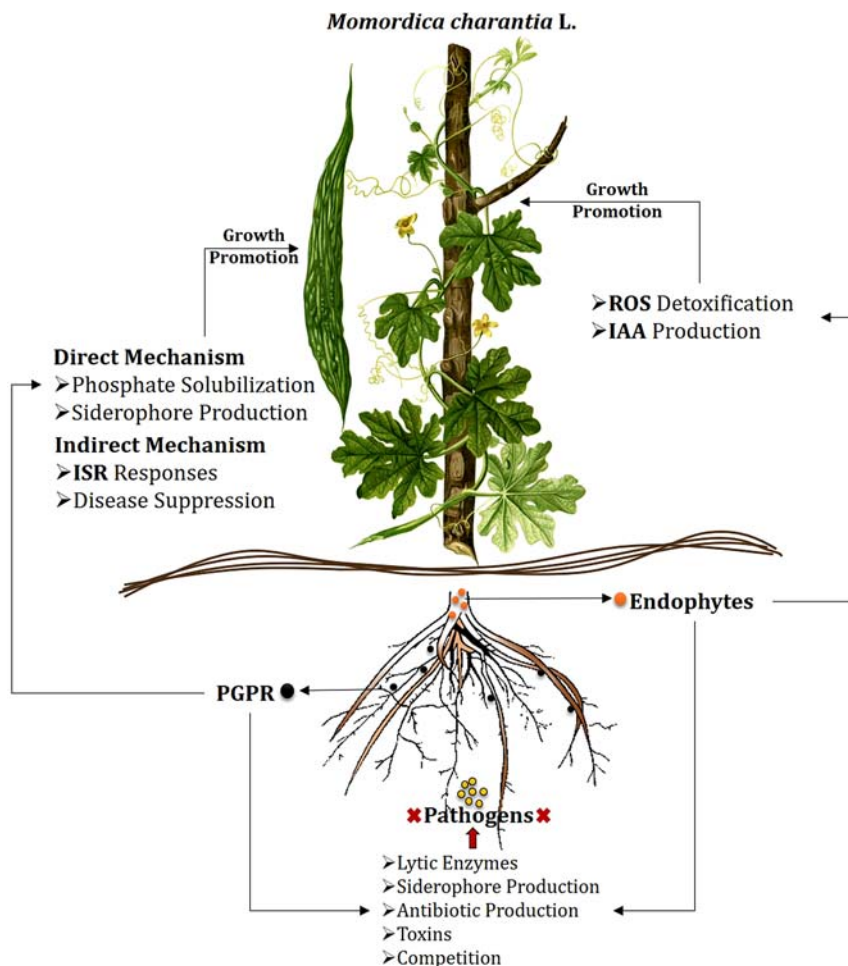


Figure 11.5 Overview of plant growth promoting bacteria (PGPB) interaction with *Momordica charantia* L.

include bacteria, fungi, and actinomycetes (Santos et al., 2012). Of total microbial load, 1%–50% of bacteria and 0.1%–0.5% of fungi species have been hypothesized to be capable of solubilizing insoluble inorganic phosphate. Both, Gram negative and Gram positive bacteria are capable of phosphate solubilization (Khan et al., 2007). In this study, P solubilization was most frequently detected in *Bacillus* and the strains of *Pseudomonas* and *Acinetobacter*. It is also reported that high population of phosphate solubilizing bacteria are commonly found in the rhizosphere in comparison to nonrhizospheric soil (Reyes and Valdúz, 2006).

Phytohormone acts as a signal molecule in the regulation of plant development. IAA production is an important trait of PGPR. The production of IAA is strain specific and also influenced by culture conditions, growth stages, and substrate availability (Mirza et al., 2001). In this study, all the rhizobacterial isolates of *M. charentia* produced IAA in the presence of tryptophan. Previously many authors already reported IAA production in different rhizobacterial species such as *Pseudomonas*, *Bacillus*, and *Acinobacter* (Kumar et al., 2014, 2016b; Glick, 2014).

Iron is the most common element on earth and is required for the growth and synthesis of plants. In natural form, iron is present in sparingly soluble ferric ion or Fe^{+3} , but it is un-utilized by both plants and microorganism (Rout and Sahoo, 2015). Bacteria synthesize siderophores molecules which have high affinity for Fe^{+3} and act as chelating agents. It binds with Fe^{+3} molecules and made them insoluble form to soluble and facilitates iron uptake by microorganisms (Hider and Kong, 2010). The siderophore-mediated competition for iron is the mechanism responsible for the antagonistic activity. The presence of iron-chelating compounds makes the bacteria better competitors for iron that prevents growth of pathogenic microorganisms. In the previous study, phosphate solubilization, IAA, and siderophore production are reported in *Bacillus*, *Azotobacter* and *Pseudomonas* sp. by (Zhao et al., 2014; Kumar et al., 2015a, 2016b). HCN production plays an important role in the biological control of pathogens (Voisard et al., 1989). Production of HCN is a strain specific PGP traits and their synthesis acts as an inducer of plant resistance. Production of ammonia is also a strain-specific and their emission alters the pH of the rhizosphere and influences microorganism diversity and plant-microbe interactions (Weise et al., 2013).

C and N substrate utilization pattern is strain specific, hierarchical cluster analysis (between groups linkage) measuring the squared Euclidean distance on the basis of source utilization pattern revealed the similarity between the substrate utilization pattern of different rhizobacterial specie. The genera showing high affinity to the same were placed nearby in the cluster (Litzner et al., 2006).

The antibiotic disc act differentially upon the growth of different bacterial strains and their sensitivity also depends upon the isolation source (Arunachalam and Gayathri, 2010; Kumar et al., 2015a, b, 2016a, b). PGPR strains produced secondary metabolites in different concentration having significant role in synthesis of antibiotics, antifungal substances, insecticides, and immunosuppressant (Grobelak et al., 2015). In present

study, the isolated bacterial strains exhibited differential response toward all the six antibiotics. Most of the strains were sensitive to Chloramphenicol (except R12), Spectinomycin (except RS11), and Kanamycin (except RS10, RS11), while Polymyxin B were the least effective as diameter wise. One-third of the strains were resistant to Erythromycin (R7, R12, RS5, and RS10) and Rifampicin (R12, RS1, and RS11). Previously, same types of trends Chloramphenicol and Erythromycin were the most sensitive and Polymyxin B least sensitive antibiotic against were found in case of rhizobacterial strains of turmeric (Kumar et al., 2016b).

Currently salinity and draught are the two major concerns of abiotic stresses that limit the growth and productivity of crop. High alkalinity in the soil adversely affects crop production and bio productivity. PGPR strains tolerate and survive in the stress conditions and alleviate the salinity stress (Saharan and Nehra, 2011; Kumar et al., 2016b). In the present study, the significant variations observed during the stress tolerance capacity especially during the salinity. All the strains tolerated 4% NaCl, but their response differed at the level of 8% of NaCl whereas, some of the strains tolerated 10% of NaCl level. Similar type of observations were reported by Kumar et al. (2016b) in case of *P. fluorescens* (8% NaCl), *Bacillus sp.* (8% NaCl) and Gayathri et al. (2010) reported *Bacillus sp.* isolated from marshy areas had been showed the tolerance capacity of 10% NaCl.

Sodium azide (NaN_3) is a strong chemo-mutagen, induced a broad variation in morphology and yield parameters in comparison to normal plant. It is a common bactericide, pesticide, and industrial nitrogen gas generator and known as a highly mutagenic in certain plants (Qurainy, 2009). The isolated strains (R7, R12, RS1, RS5, and RS14) found resistant to 0.2% of NaN_3 concentration during the tolerance activity. It has been reported that concentration ranged (1×10^{-3} to 5×10^{-3} M) affect wide range of variation in plant properties (plant height, leaf area, fresh, and dry weight) during the seedlings developed from treated seeds with sodium azide (Qurainy, 2009).



11.5 CONCLUSION

Present studies revealed diverse bacterial species in the rhizosphere of *Momordica charantia*. *Bacillus* is the most dominant bacterial species

among the 12 isolates; however, all the isolated strains showed variable multiplant growth promotion responses in terms of plant growth promoting traits analysis such as IAA and ammonia production, substrate utilization patterns, and antibiotic sensitivity test, which may be used as plant or soil inoculants for the better environment management and sustainable agriculture. Moreover, some strains were capable to tolerate NaCl concentration (10%), suggesting their future application in the salinity and draught management.

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REFERENCES

- Aarons, S., Abbas, A., Adams, C., Fenton, A., O'Gara, F., 2000. A regulatory RNA (Prrb RNA) modulates expression of secondary metabolite genes in *Pseudomonas fluorescens* F113. *J Bacteriol*, 182, 3913–3919.
- Arunachalam, C., Gayathri, P., 2010. Studies on bioprospecting of endophytic bacteria from the medicinal plant of *Andrographis paniculata* for their antimicrobial activity and antibiotic susceptibility. *Int J Curr Pharm Res*, 2 (4), 63–68.
- Bakare, R.I., Magbagbeola, O.A., Akinwande, A.I., Okunowo, O.W., 2010. Nutritional and chemical evaluation of *Momordica charantia*. *J Med Plant Res*, 4 (21), 2189–2193.
- Bergey's Manual of Determinative Bacteriology, ninth ed., 1994. Sneath P.H.A., Staley J. T., Williams S.T. (Eds.), Williams and Wikkins, Baltimore, USA.
- Brick, J.M., Bostock, R.M., Silverstone, S.E., 1991. Rapid in situ assay for indole acetic acid production by bacteria immobilized on nitrocellulose membrane. *Appl Environ Microbiol*, 57, 535–538.
- Cappuccino, J.G., Sherman, N., 1992. Biochemical activities of microorganisms. Microbiology, A Laboratory Manual. The Benjamin/Cummings Publishing Co, California, USA.
- Garbeva, P., Veen, J.A., Elsas, J.D., 2003. Predominant *Bacillus* spp. in agricultural soil under different management regimes detected via PCR-DGGE. *Microb Ecol*, 45, 302–316.
- Gayathri, S., Saravanan, D., Radhakrishnan, M., Balagurunathan, R., Kathiresan, K., 2010. Bioprospecting potential of fast growing endophytic bacteria from leaves of mangrove and salt-marsh plant species. *Indian J Biotech*, 9 (4), 397–402.
- Glick, B.R., 2012. Plant growth-promoting bacteria: Mechanisms and application. *Scientifica*, Article ID963401 (15) page.
- Glick, B.R., 2014. Bacteria with ACC deaminase can promote plant growth and help to feed the world. *Microbiol Res*, 169, 30–39.
- Grobelak, A., Napora, A., Kacprzak, M., 2015. Using plant growth promoting rhizobacteria (PGPR) to improve plant growth. *Eco Eng*, 84, 22–28.
- Hider, R.C., Kong, X., 2010. Chemistry and biology of siderophores. *Nat Prod Rep*, 27 (5), 637–657.
- Jasim, B., Joseph, A.A., John, C.J., Mathew, J., Radhakrishnan, E.K., 2014. Isolation and characterization of plant growth promoting endophytic bacteria from the rhizome of *Zingiber officinale* 3. *Biotech*, 4 (2), 197–204.

- Joseph, B., Jini, D., 2013. Antidiabetic effects of *Momordica charantia* (bitter melon) and its medicinal potency. *Asian Pac J Trop Dis*, 3 (2), 93–102.
- Khan, M.S., Zaidi, A., Wani, P.A., 2007. Role of phosphate-solubilizing microorganisms in sustainable agriculture – A review. *Agron Sustain Develop*, 27, 29–43.
- Kloepper, J.W., Leong, J., Teintze, M., Schroth, M.N., 1980. Enhanced plant growth by Siderophore produced by plant growth promoting rhizobacteria. *Nature*, 286, 885–886.
- Kumar, A., Singh, R., Giri, D.D., Singh, P.K., Pandey, K.D., 2014. Effect of *Azotobacter chroococcum* CL13 inoculation on growth and curcumin content of turmeric (*Curcuma longa* L.). *Int J Curr Microbiol App Sci*, 3 (9), 275–283.
- Kumar, A., Vandana, Yadav, A., Giri, D.D., Singh, P.K., Pandey, K.D., 2015b. Rhizosphere and their role in plant – microbe interaction. In: Kishore Chaudhary, K., Wattal Dhar, D. (Eds.), *Microbes in Soil and Their Agricultural Prospects*. Nova Science Publisher, Inc, Hauppauge, NY, pp. 83–97.
- Kumar, A., Singh, R., Yadav, A., Giri, D.D., Singh, P.K. and, Pandey, K.D. (Eds.), 2016a. Isolation and characterization of bacterial endophytes of *Curcuma longa* L. 3 *Biotech*, 6, 60.
- Kumar, A., Vandana, Singh, M., Singh, P.P., Singh, S.K., Singh, P.K., et al., 2016b. Isolation of plant growth promoting rhizobacteria and their impact on growth and curcumin content in *Curcuma longa* L. *Biocat Agri Biotech*, 8, 1–7.
- Kumar, K.S.N., Sowmyamala, B.V., Kumar, P.G.S., Vasudev, P.N., Kumar, R.V., Nagaraj, H.T., 2013. Effect of plant growth promoting rhizobacteria (PGPR) on growth and yield of bitter gourd. *Int J Appl Biol Pharm Tech*, 3 (1), 1–7.
- Kumar, V., Kumar, A., Pandey, K.D. and, Roy, B.K. (Eds.), 2015a. Isolation and characterization of bacterial endophytes from the roots of *Cassia tora* L. *Ann Microbiol*, 65, 1391–1399.
- Laslo, E., Gyorgy, E., Mara, G., Tamas, E., Abraham, B. and, Lanyi, S. (Eds.), 2012. Screening of plant growth promoting rhizobacteria as potential microbial inoculants. *Crop Prot* 40, 43–48.
- Litzner, B.R., Caton, T.M., Schneegurt, M.A., 2006. Carbon utilization, antibiotic sensitivity and numerical taxonomy of bacterial isolate from the great salt plains of Oklahoma. *Arch Microbiol*, 85, 286–296.
- Lorck, H., 1948. Production of hydrocyanic acid by bacteria. *Physiol Plant*, 1, 142–146.
- Maier, R.M., Pepper, I.L. and, Gerba, C.P. (Eds.), 2009. *Environmental Microbiology*, second ed. Academic Press, London.
- Mirza, M.S., Ahmad, W., Latif, F., Haurat, J., Bally, R., Normand, P., et al., 2001. Isolation, partial characterization, and the effect of plant growth-promoting bacteria (PGPB) on micro-propagated sugarcane in vitro. *Plant Soil*, 237, 47–54.
- Morgan, J.A.W., Bending, G.D., White, P.J., 2005. Biological costs and benefits to plant-microbe interactions in the rhizosphere. *J Exp Bot*, 56 (417), 1729–1739.
- Okon, Y., Labandera-Gonzalez, C.A., 1994. Agronomic applications of *Azospirillum*. In: Ryder, M.H., Stephens, P.M., Bowen, G.D. (Eds.), (Eds.), *Improving Plant Productivity with Rhizosphere Bacteria*. Commonwealth Scientific and Industrial Research Organization, Adelaide, Australia, pp. 274–278.
- Oku, S., Komastu, A., Tajima, T., Nakashimada, Y., Kato, J., 2012. Identification of chemotaxis sensory proteins for aminoacids in *Pseudomonas fluorescens* Pf0-1 and their involvement in chemo taxis to tomato root exudates and root colonization. *Microbes. Environ.* 27 (4), 462–469.
- Ozusaglam, M.A., Karakoca, K., 2013. Antimicrobial and antioxidant activities of *Momordica charantia* from Turkey. *Afr J Biotech*, 12 (13), 1548–1558.
- Qurainy, F., 2009. Effects of sodium azide on growth and yield traits of *Eruca sativa* L. *World Appl Sci J*, 7 (2), 220–226.

- Reyes, V.A., Valduz, Z., 2006. Phosphate solubilising microorganisms isolated from the rhizospheric and bulk soils of colonizer plants at an abandoned rock phosphate mine. *Plant Soil*, 287, 69–75.
- Rout, G.R., Sahoo, S., 2015. Role of iron in plant growth and metabolism. *Rev Agric Sci*, 3, 1–24. Available from: <https://doi.org/10.7831/ras.3.1>.
- Saharan, B.S., Nehra, V., 2011. Plant growth promoting rhizobacteria: a critical review. *Life Sciences and Medicine Research LSMR*, 21.
- Santos, E.A., Dos Ferreira, L.R., Costa, M.D., Silva, M.C., Reis, M.R., França, A.C., 2012. Occurrence of symbiotic fungi and rhizospheric phosphate solubilization in weeds. *Agron*, 35, 49–55.
- Schwyn, B., Neilands, J.B., 1987. Universal chemical assay for detection and determination of siderophore. *Anal Biochem*, 160, 47–56.
- Shukla, P.N., 2009. Diversity and Characteristics of Methylootrophs from a Degraded Coal Mine Area. Banaras Hindu University, p. 34.
- Singh, R., Kumar, A., Bhuvaneshwari, K., Pandey, K.D., 2012. Gas chromatography-mass spectrometry analysis and photo-chemical screening of methanolic fruit extract of *Momordica charantia*. *J Recent Adv Agri*, 1 (4), 122–127.
- Voisard, C., Keel, C., Haas, D., Defago, G., 1989. Cyanide production by *Pseudomonas fluorescens* helps suppress black root rot of tobacco under gnotobiotic conditions. *EMBO J*, 8, 351–358.
- Weise, T., Kai, M., Piechulla, B., 2013. Bacterial ammonia causes significant plant growth inhibition. *PLoS One*, 8 (5), e63538.
- Wieland, G., Neumann, R., Backhaus, H., 2001. Variation of microbial communities and rhizoplane in response to crop species, soil type and crop development. *Appl Environ Microbiol*, 67, 5849–5854.
- Zhao, K., Penttinen, P., Zhang, X., Ao, X., Liu, M., Yu, X., et al., 2014. Maize rhizosphere in Sichuan, China, hosts plant growth promoting *Burkholderia cepacia* with phosphate solubilizing and antifungal abilities. *Microbiol Res*, 169 (1), 76–82.

Tolerance of Heavy Metal Toxicity Using PGPR Strains of *Pseudomonas Species*

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12.1 INTRODUCTION

Accumulation of heavy metals is one of the severe environmental concern that poses many adverse impacts on the human health, plant, and soil. In the normal concentration, metals constitute an essential nutritional requirement for all the living organisms due to their participation in a wide variety of biological activities such as enzymatic cofactors, proteins and in stimulator or biological pathways and play a key role in the attainment of metal homeostasis (da Silva et al., 1991; Appanna et al., 1995). The metals, having specific weight more than 5.0 g/cm³, are defined as heavy metals and categorized into three different classes: Toxic metals (e.g., Hg, Cr, Pb, Zn, Cu, Ni, Cd, As, Co, Sn, etc.), precious metals (e.g., Pd, Pt, Ag, Au, Ru, etc.), and radionuclides (e.g., U, Th, Ra, Am, etc.) (Nies, 1999; Bishop, 2002; Ahemad, 2014).

Currently, the levels of some heavy metals in the environments are increasing, and it reaches up to the level of toxicity. The industrialization of fertiliser, pesticide, metallurgy industry, combustion of fossil fuels, directly or indirectly release vast amounts of toxic metals into the environment resulting hazardous impacts on both ecological and human health (Chibuike and Obiora, 2014; Carlos et al., 2016). It has been estimated that the anthropogenic industries and domestic sources enhance the emissions level of lead, cadmium, vanadium, and zinc up to 100-fold.

The exceeding level of these heavy metals poses severe health and ecological risks (Wang and Chen, 2006; Kotrba et al., 2009; Gomathy and Sabarinathan, 2010; Ahemad and Malik, 2011).

The toxicity of heavy metal is a great concern for the environmentalists due to very low degradability and high persistence capacity in the environment (Ahemad and Ashraf, 2012). Metal toxicity varies according to their concentration or nature of the element. Some of the heavy metals display toxicity even at the frequency of $1\text{--}10\text{ mg l}^{-1}$. The ions of the elements Hg and Cd show toxicity at the concentration of $0.001\text{--}0.1\text{ mg l}^{-1}$, whereas some elements change their nature in the changing environment (Alkorta et al., 2004; Wang and Chen, 2006; Rajkumar et al., 2012; Carlos et al., 2016).



12.2 HEAVY METALS AND THEIR EFFECTS ON PLANT GROWTH

Excess accumulation of some heavy metals in the soil adversely affect the texture and productivity of soil, growth, yields, nutrient availability in the plants, microbial community and also the health-related issues of humans (Jing et al., 2005; López-Millán et al., 2009; Gupta et al., 2013; Etesami, 2018). In some cases, a higher concentration of metals enhanced malondialdehyde (MDA), superoxide dismutase (SOD), and peroxidase (POD) activities which induce an oxidative stress response in plants at $(1\text{--}10\text{ }\mu\text{m})$.

Lead (Pb) forms different types of complexes with soil components and some part of Pb concentration absorbed by plants mainly through the roots. Excessive accumulation of lead in the tissue impairs various morphological, physiological, and biochemical functions of plants. It causes phytotoxicity by changing cell membrane permeability and also by reacting active groups of multiple enzymes involved in plant metabolism. Pb toxicity strongly inhibits seed germination, root elongation, seedling development, plant growth, transpiration, chlorophyll production, and water and protein content. The higher concentration of Pb also affects plant performance, fruits and dry weights of plant, as reported in case of tomato (Balba et al., 1991; Opeolu et al., 2010).

Nickel (Ni) concentration in the plants limits growth and metabolism of plants. Deficiency of Ni causes reduced growth, induction of senescence, leaf chlorosis, alterations in N metabolism, and reduced Fe uptake.

Although Ni is metabolically vital in plants, it is toxic to the plant in excessive amounts in the soil. High concentrations of Ni retards seed germinability, shoot and root growth, deform plant parts, abnormal flower shape, induce leaf spotting, and decrease biomass production. Palacios et al. (1998) studied the effect Ni on the nutrition of tomato plants receiving 5, 15, and 30 mg Ni l⁻¹, grown in a nutrient solution and reported that presence of Ni in nutrient medium negatively affected plant growth, decreasing dry matter yield compared to control plants. Copper (Cu) is also an essential micronutrient metal required for the normal functioning of plants. Accumulation of excess Cu has a detrimental effect on plant growth and primarily affects the morphology and growth of the root (Marschner, 1995; Sheldon and Menzies, 2005).

Mercury (Hg) is one of the highly toxic elements tend to accumulate in the roots and in moderate amounts in the stems of plants (Lenka et al., 1992; Dushenkov et al., 1995). Exposure to Hg can also reduce photosynthesis, transpiration rate, water uptake, and chlorophyll synthesis. Both organic and inorganic Hg has been showed to cause loss of potassium, magnesium, manganese and accumulation of iron (Boening, 2000).

To mitigate heavy metals contamination from the soil or environment, various physicochemical approaches including chemical precipitation, chemical oxidation, reduction, ion exchange, filtration, and electrochemical treatment have been proposed (Vijayaraghavan et al., 2006). However, these processes adversely affect the health and environment of soil, plant as well as humans. Therefore, in this context, plant growth promoting bacteria (PGPB) is considered as one of the best-suited choices for the mitigation of all these heavy metals problems due to environmentally safe and less adverse effect (Whipps, 2001; Idris et al., 2004; Richardson et al., 2009; Rajkumar et al., 2012).



12.3 *PSEUDOMONAS* SP. IN HEAVY METAL TOLERANCE

Pseudomonas is the bacterial genus of gram-negative, Gamma proteobacteria, belonging to the family Pseudomonadaceae containing more than 191 species. From the last two decades, *Pseudomonas* is extensively used in sustainable agriculture as biocontrol, plant growth promoting agents, and in the management of biotic and abiotic stress (Walsh et al., 2001; Pathma et al., 2011; Jain and Pandey, 2016; Kumar et al., 2017a,b).

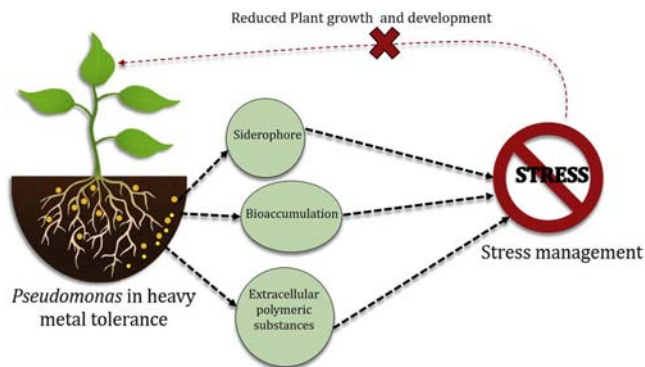


Figure 12.1 Mechanism of *Pseudomonas* in heavy metal tolerance.

Pseudomonas is one of the most diversified bacterial genera present in the soil, water, plants, etc. The rhizosphere of the plant is the most prominent ecological niche for the *Pseudomonas* in comparison to the bulk soil. Root exudates of the plant interact gram-negative bacteria more preferably than gram-positive bacterial strains due to cell wall compositions and root exudates composition (Kumar et al., 2015a,b). The modes of action of *Pseudomonas* are same as other PGPB. The principal mechanism of bio-control, growth promotion, and stress management includes stimulating phytohormones, production of ammonia, siderophores, and HCN, solubilization of phosphate, bioaccumulation, and secretions of extracellular polymeric substances (EPSs) (Kumar et al., 2015a,b, 2016a,b; Oaikhena et al., 2016; Mishra et al., 2017). Many *Pseudomonas* sp. have such tolerance capacities that even survive in highly toxic conditions. Appanna et al. (1996) reported strains of *Pseudomonas fluorescens* could survive in the presence of either Mn, CO, or Cs media with multiple-metal stress by immobilizing toxic elements. Another study of Zhang et al. (2012), reported cadmium-resistant *Pseudomonas aeruginosa* having multiple heavy-metals tolerance capacity. This bacterium could serve as an effective metal sequestering and growth-promoting bioinoculant for plants grown in metal-contaminated soil (Fig. 12.1).



12.4 MECHANISM OF HEAVY METAL TOLERANCE

Bioavailability of heavy metals in the soil causes the toxicity and adversely affects the plant growth. Interactions of heavy metals with

specific PGPR tackle the problems of toxicity and plant growth promotion (Fig. 12.2). By altering the physicochemical properties of soil to enhance metal bioavailability could trigger the mitigation, detoxification, or removal of heavy metals from soil (Mishra et al., 2017). Several reports have articulated the use of Plant growth promoting rhizobacteria (PGPR) to efficiently remove heavy metals from the contaminated system by biosorption (François et al., 2012; Velásquez and Dussan, 2009) and bioaccumulation mechanisms (Velásquez and Dussan, 2009).

Pseudomonas directly or indirectly influences the metals availability and accumulation in the environment and play an important role in decreasing existing contamination. In response to metals in the environment, microorganisms are endowed with numerous mechanisms for the detoxification and resistance from the adverse effect of heavy metals via demobilization, mobilization, precipitation, biosorption, bioaccumulation, and biogeochemical cycling. Currently, many different strains of *Pseudomonas* have

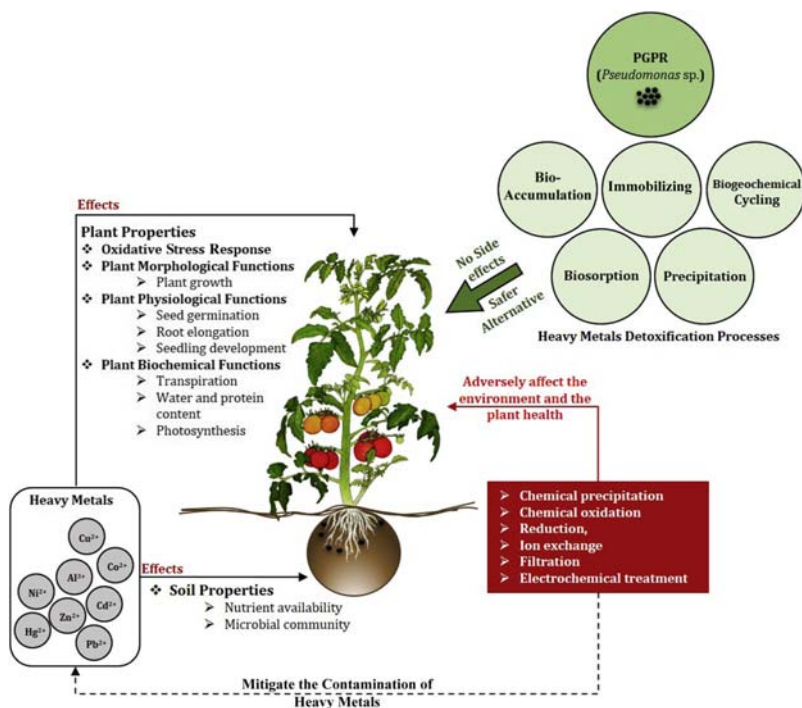


Figure 12.2 Overview of heavy metal tolerance in plants via plant growth promoting bacteria.

Microbial communities in the rhizospheric zone of plant secrete extra-cellular polymeric substances (EPS) such as polysaccharides, lipopolysaccharides, proteins, etc. having an anionic functional group, which help to remove metals from the rhizosphere through the process of biosorption (Ayangbenro and Babalola, 2017; Mishra et al., 2017). Production of EPSs is one of the characteristic features of most of the *Pseudomonas sp.* (Wingender et al., 2001). The characteristics of **(EPSs)** are the mobilization or immobilization of toxic metals by binding with the heavy metals such as lead, cadmium, and uranium. Interactions with the ions of heavy metals are the direct consequence of the binding with negatively charged functional groups of the extra polymer substances. These groups include phosphate, hydroxyl, succinyl, and uronic acids. EPS produced by some microbes induce biofilm formation in response to the exposure of toxic heavy metal. The formation of biofilm help in detoxification of heavy metals by enhancing the tolerance capacity of a microbial cell or by transforming toxic metal ions to nontoxic metals (Gupta and Diwan, 2016).

Siderophores are low-molecular-weight chelating agents (200–2000 Da) produced by bacteria, fungi and plants to facilitate uptake of iron (Chu et al., 2010; Hider and Kong, 2010). Iron is one of the essential elements required for the development and normal functioning of living organisms. Siderophores have a variety of chemical structures, which possess electron rich atoms such as oxygen or nitrogen electron donor atoms that can bind with metal cations (Chu et al., 2010; Hider and Kong, 2010).

Siderophores are either polycarboxylates or commonly present bacterial catecholate. In case of *Pseudomonas* siderophore secretes pseudobactin and pyoverdine, both carry hydroxamate and catecholate groups, which act as chelating agent (Abdallah and Pattus, 2000; Boukhalfa and Crumbliss, 2002).

In a study Hannauer et al. (2010) and Hernlem et al. (1996) carried out a study with 16 different metals (Ag^+ , Al^{3+} , Cd^{2+} , Co^{2+} , Cr^{2+} , Cu^{2+} , Eu^{3+} , Ga^{3+} , Hg^{2+} , Mn^{2+} , Ni^{2+} , Pb^{2+} , Sn^{2+} , Tb^{3+} , Tl^+ , and Zn^{2+}) and concluded that siderophores pyoverdine and pyochelin produced by *P. aeruginosa*, are able to chelate all these metals (Braud et al., 2009a,b).

This study revealed that toxic metals induce production of some siderophores and these chelators may play important role in bacterial heavy metal tolerance. During detoxification toxic metals enter the periplasm of gram-negative bacteria mostly by diffusion across the porins of membrane

and thus (Lutkenhaus, 1977; Pugsley and Schnaitman, 1978). Thus, the binding of metals to siderophores in the extracellular medium reduces the free metal concentration, probably affecting diffusion which ultimately decrease their toxicity (Braud et al., 2010). The biological function of siderophore is also concentrate iron from environments where concentration is low and to facilitated into the cell (Kloepper et al., 1980; Kumar et al., 2016a,b). The mitigation capacity of toxic metals depends upon adsorption capacity of receptor and metal-siderophore (Huyer and Page, 1988). Siderophore formation in response to heavy metal exposure may have both beneficial and detrimental effects. It may provide protective effects by lowering the free metal concentration or disturbs the receptor against the metal-siderophore complex. The presence of metals other than iron is known to stimulate siderophore formation in a number of bacteria and fungi (Winkelmann and Zahner, 1973; Huyer and Page, 1988). Zhang et al. (2012) reported metal tolerance capacity of siderophore producing *P. aeruginosa* was positively related with metal tolerance capacity.

Bioaccumulation/biosorption of heavy metals by microbial cell has been recognized as potential physico-chemical methods for the removal of heavy metal ions (Hussein et al., 2004). Bioaccumulation is responsible for the uptake and detoxification of heavy metals. Generally, bioaccumulation consist of two process, first one is “passive process” in which there is no any involvement of any metabolism, simply “biosorption,” second one is “active process” in which involvement of metabolism and energy taken place for the transport and bioaccumulation of metals and it is characteristics of living organism (Gutiérrez-Corona et al., 2016; Mishra et al., 2017). Many PGPR alter metal bioavailability in the soil through acidification, chelation, complexation, precipitation, and redox reactions. Metal accumulation or chelation involves some specific metal binding peptides. Metallothioneins and glutathione-derived peptides secreted by PGPR including *Pseudomonas*, fungi and also by plant in response to metal toxicity and resulting deposition of excess or heavy metals in the plant or microbial cell (Miransari, 2011, Mishra et al., 2017). Bioavailability and adsorption of heavy metals in the rhizosphere favor in acidic pH conditions (Merdy et al., 2009; Mishra et al., 2017). The Organic acids released by microbes’ lower soil pH, which is responsible for the sequester of soluble metal ions (Turnau and Kottke, 2005). Metal bioavailability can be influenced by metabolic by-products that result in metal reduction. During bioaccumulation, soluble metals reduced to less soluble metal salts

or precipitates. Most studies indicating biosorption for metal removal involved the use of either laboratory-grown microorganism or biomass generated by the pharmacology and food processing industries or wastewater treatment units. Hussein et al. (2004) used *Pseudomonas* sp. as a potential bio sorbent for the removal of heavy metals in ground water and found *Pseudomonas* sp. with 81% degrading capacity of heavy metals. In another study, Appanna et al. (1995) examined the impact of multiple-metal stress on the soil bacterium *P. fluorescens* and concluded tolerance to millimolar quantities of aluminum, iron, calcium, zinc, and gallium is mediated by the elaboration of exocellular phosphatidyl ethanolamine.



12.5 FUTURE PROSPECTIVE

In the current scenario of climate change and shrinkage of agricultural lands, there is an urgent need to search the new alternatives for of tolerance or detoxification of heavy metals. PGPR (rhizospheric and endophytic microorganisms) are one of the best suited choices, in the development of phytoremediation techniques and has to be elucidating to speed up the mitigation processes. Currently, many endophytic strains are being used as plant and soil inoculants as biocontrol or plant growth promoting agents but limited information is available on PGPR and endophytic mediated heavy metal tolerance. Furthermore, there is need to study the interaction of plant-microbes—heavy metals and their impact on the plant interior to access their biochemical and physiological aspects. Moreover, the detoxifying trait of this resistant *Pseudomonas* strain can be exploited to develop heavy metal resist consortia using the omics technology (genomics, proteomics, and transcriptomics). Despite of broad spectrum of *Pseudomonas* in the field of growth promotion, disease management, or stress management, researchers must focus on the novel strains and also imply them in the biocontrol, growth promotion, or stress management in plants.

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REFERENCES

- Abdallah, M.A., Pattus, F., 2000. Siderophores and iron transport in microorganisms. *J Chin. Chem. Soc.* 47, 1–20.
- Ahemad, M., 2014. Remediation of metalliferous soils through the heavy metal resistant plant growth promoting bacteria: paradigms and prospects. *Arab J Chem.* Available from: <https://doi.org/10.1016/j.arabjc.2014.11.020>.
- Ahemad, M., Malik, A., 2011. Bioaccumulation of heavy metals by zinc resistant bacteria isolated from agricultural soils irrigated with wastewater. *Bacteriol J*, 2, 12–21.
- Ahemad, M.S.A., Ashraf, M., 2012. Essential roles and hazardous effects of nickel in plants. In: Whitacre, D. (Ed.), *Reviews of Environmental Contamination and Toxicology (Continuation of Residue Reviews)*, vol. 214, Springer, New York, pp. 125–167.
- Alkorta, I., Hernández-Allica, Becerril, J.M., Amezcaga, I., Albizu, I., Garbisu, C., 2004. Recent findings on the phytoremediation of soils contaminated with environmentally toxic heavy metals and metalloids such as zinc, cadmium, lead, and arsenic. *Rev Environ Sci Biotechnol* 3, 71–90.
- Appanna, V.D., Finn, H., Pierre, M.S., 1995. Exocellular phosphatidylethanolamine production and multiple-metal tolerance in *Pseudomonas fluorescens*. *FEMS Microbiol Lett* 131 (1), 53–56.
- Appanna, V.D., Gázsó, L.G., Pierre, M.S., 1996. Multiple-metal tolerance in *Pseudomonas fluorescens* and its biotechnological significance. *J Biotechnol*, 52 (2), 75–80.
- Ayangbenro, A.S., Babalola, O.O., 2017. A new strategy for heavy metal polluted environments: a review of microbial biosorbents. *Int J Environ Res Public Health* 14 (1), 94.
- Balba, A., Shibiny, G., El-Khatib, E., 1991. Effect of lead Increments on the yield and lead content of tomato plants. *Water Air Soil Pollut*, 57–58, 93–99.
- Bishop, P.L., 2002. *Pollution Prevention: Fundamentals and Practice*. Tsinghua University Press, Beijing, China.
- Boening, D.W., 2000. Ecological effects, transport, and fate of mercury: a general review. *Chemosphere*, 40 (12), 1335–1351.
- Bopp, L.H., Ehrlich, H.L., 1998. Chromate resistance and reduction in *Pseudomonas fluorescens* strain LB300. *Arch Microbiol*, 150 (5), 426–431.
- Boukhalfa, H., Crumbliss, A.L., 2002. Chemical aspects of siderophore mediated iron transport. *Biometals* 15, 325–339.
- Braud, A., Hannauer, M., Milsin, G.L.A., Schalk, I.J., 2009a. The *Pseudomonas aeruginosa* pyochelin-iron uptake pathway and its metal specificity. *J. Bacteriol.* 191, 5317–5325.
- Braud, A., Hoegy, F., Jezequel, K., Lebeau, T., Schalk, I.J., 2009b. New insights into the metal specificity of the *Pseudomonas aeruginosa* pyoverdine-iron uptake pathway. *Environ. Microbiol.* 11, 1079–1091.
- Braud, A., Geoffroy, V., Hoegy, F., Mislin, G.L.A., Schalk, I.J., 2010. The siderophores pyoverdine and pyochelin are involved in *Pseudomonas aeruginosa* resistance against metals: Another biological function of these two siderophores. *Environ. Microbiol. Rep.* 2, 419–425.
- Carlos, M.H.J., Stefani, P.V.Y., Janette, A.M., Melani, M.S.S., Gabriela, P.O., 2016. Assessing the effects of heavy metals in ACC deaminase and IAA production on plant growth-promoting bacteria. *Microbiol Res* 188, 53–61.
- Chen, X.C., Wang, Y.P., Lin, Q., Shi, J.Y., Wu, W.X., Chen, Y.X., 2005. Biosorption of copper (II) and zinc (II) from aqueous solution by *Pseudomonas putida* CZ1. *Colloids Surf B Biointerfaces* 46 (2), 101–107.
- Chibuike, G.U., Obiora, S.C., 2014. Heavy metal polluted soils: effect on plants and bioremediation methods. *Appl Environ Soil Sci*, 2014(2014) 1–12.

- Choudhary, S., Sar, P., 2009. Characterization of a metal resistant *Pseudomonas* sp. isolated from uranium mine for its potential in heavy metal (Ni $2+$, Co $2+$, Cu $2+$, and Cd $2+$) sequestration. *Bioresour Technol* 100 (9), 2482–2492.
- Chu, B.C., Garcia-Herrero, A., Johanson, T.H., Krewulak, K.D., Lau, C.K., Peacock, R. S., et al., 2010. Siderophore uptake in bacteria and the battle for iron with the host; a bird's eye view. *Biomaterials* 23, 601–611.
- Cooksey, D.A., 1990. Plasmid-determined copper resistance in *Pseudomonas syringae* from impatiens. *Appl Environ Microbiol* 56 (1), 13–16.
- da Silva, Frausto, J.J.R., Williams, R.J.P., 1991. The Biological Chemistry of the Elements. Oxford University Press, Oxford, pp. 3–22.
- Dushenkov, V., Kumar, P.B.A.N., Motto, H., Raskin, I., 1995. Rhizofiltration: the use of plants to remove heavy metals from aqueous streams. *Environ Sci Technol*, 29, 1239–1245.
- Etesami, H., 2018. Bacterial mediated alleviation of heavy metal stress and decreased accumulation of metals in plant tissues: mechanisms and future prospects. *Ecotoxicol Environ Saf*, 147, 175–191.
- François, F., Lombard, C., Guigner, J.M., Soreau, P., Brian-Jaisson, F., Martino, G., et al., 2012. Isolation and characterization of environmental bacteria capable of extracellular biosorption of mercury. *Appl Environ Microbiol*, 78 (4), 1097–1106.
- Gomathy, M., Sabarinathan, K.G., 2010. Microbial mechanisms of heavy metal tolerance: a review. *Agric Rev*, 31 (2), 133–138.
- Gutiérrez-Corona, J.F., Romo-Rodríguez, P., Santos-Escobar, F., Espino-Saldana, A.E., Hernández-Escoto, H., 2016. Microbial interactions with chromium: basic biological processes and applications in environmental biotechnology. *World J. Microbiol. Biotechnol.* 32 (12), 191.
- Gupta, D.K., Huang, H.G., Corpas, F.J., 2013. Lead tolerance in plants: strategies for phytoremediation. *Environ Sci Pollut Res*, 20 (4), 2150–2161. 1.
- Gupta, P., Diwan, B., 2016. Bacterial Exopolysaccharide mediated heavy metal removal: a review on biosynthesis, mechanism and remediation strategies. *Biotechnol Rep*, 13, 58–71.
- Haefeli, C., Franklin, C.H., Hardy, K., 1984. Plasmid-determined silver resistance in *Pseudomonas stutzeri* isolated from a silver mine. *J Bacteriol*, 158 (1), 389–392.
- Hassen, A., Saidi, N., Cherif, M., Boudabous, A., 1998a. Resistance of environmental bacteria to heavy metals. *Bioresour Technol* 64 (1), 7–15.
- Hannauer, M., Barda, Y., Mislin, G.L., Shanzer, A., Schalk, I.J., 2010. The ferrichrome uptake pathway in *Pseudomonas aeruginosa* involves an iron release mechanism with acylation of the siderophore and a recycling of the modified desferrichrome. *J. Bacteriol.* 192, 1212–1220.
- Hassen, A., Saidi, N., Cherif, M., Boudabous, A., 1998b. Effects of heavy metals on *Pseudomonas aeruginosa* and *Bacillus thuringiensis*. *Bioresour Technol*, 65 (1), 73–82.
- Hernim, B.J., Vane, L.M., Sayles, G.D., 1996. Stability constants for complexes of the siderophore desferrioxamine B with selected heavy metal cations. *Inorg. Chim. Acta* 244, 179–184.
- Hider, R.C., Kong, X., 2010. Chemistry and biology of siderophores. *Nat. Prod. Rep.* 27, 637–657.
- Hu, M.Z.C., Reeves, M., 1997. Biosorption of uranium by *Pseudomonas aeruginosa* strain CSU immobilized in a novel matrix. *Biotechnol Prog*, 13, 60–70.
- Hu, N., Zhao, B., 2007. Key genes involved in heavy-metal resistance in *Pseudomonas putida* CD2. *FEMS Microbiol Lett*, 267 (1), 17–22.
- Hussein, H., Ibrahim, S.E., Kandeel, K., Moawad, H., 2004. Biosorption of heavy metals from waste water using *Pseudomonas sp.* *Electron J Biotechnol* 7 (1), 30–37.

- Hussein, H., Farag, S., Kandil, K., Moawad, H., 2005. Tolerance and uptake of heavy metals by *Pseudomonads*. *Process Biochem* 40 (2), 955–961.
- Huyer, M., Page, W.J., 1988. Zn²⁺ increases siderophore production in *Azotobacter vinelandii*. *J Appl Environ Microbiol*, 54 (11), 2625–2631.
- Idris, R., Trifonova, R., Puschenreiter, M., Wenzel, W.W., Sessitsch, A., 2004. Bacterial communities associated with flowering plants of the Ni hyperaccumulator *Thlaspi goesingense*. *Appl Environ Microbiol*, 70, 2667–2677.
- Jain, R., Pandey, A., 2016. A phenazine-1-carboxylic acid producing polyextremophilic *Pseudomonas chlororaphis* (MCC2693) strain, isolated from mountain ecosystem, possesses biocontrol and plant growth promotion abilities. *Microbiol Res*, 190, 63–71.
- Jing, D., Fei-bo, W.U., Guo-ping, Z., 2005. Effect of cadmium on growth and photosynthesis of tomato seedlings. *J Zhejiang Univ Sci B* 6 (10), 974–980. 1.
- Kloepper, J.W., Leong, J., Teintze, M., Schroth, M.N., 1980. *Pseudomonas* siderophores: a mechanism explaining disease suppressive soils. *Curr Microbiol*, 4, 317–320.
- Kotrba, P., Najmanova, J., Macek, T., Ruml, T., Mackova, M., 2009. Genetically modified plants in phytoremediation of heavy metal and metalloid soil and sediment pollution. *Biotechnol Adv*, 27 (6), 799–810.
- Kumar, A., Vandana, S., Yadav, A., Giri, D.D., Singh, P.K., Pandey, K.D., 2015a. Rhizosphere and their role in plant–microbe interaction. *Microbes in Soil and Their Agricultural Prospects*. Nova Science Publisher, Inc, Hauppauge, pp. 83–97.
- Kumar, A., Singh, R., Yadav, A., Giri, D.D., Singh, P.K., Pandey, K.D., 2016a. Isolation and characterization of bacterial endophytes of *Curcuma longa* L. *3 Biotech*, 6, 60.
- Kumar, A., Singh, V., Singh, M., Singh, P.P., Singh, S.K., Singh, P.K., et al., 2016b. Isolation of plant growth promoting rhizobacteria and their impact on growth and curcumin content in *Curcuma longa* L. *Biocatal Agric Biotechnol*, 8, 1–7.
- Kumar, A., Verma, H., Singh, V.K., Singh, P.P., Singh, S.K., Ansari, W.A., et al., 2017a. Role of *Pseudomonas* sp. in sustainable agriculture and disease management. In: Meena, V.S., Mishra, P.K., Bisht, J.K., Pattanayak, A. (Eds.), *Agriculturally Important Microbes for Sustainable Agriculture*. Springer, Singapore, pp. 195–215.
- Kumar, A., Singh, A.K., Kaushik, M.S., Mishra, S.K., Raj, P., Singh, P.K., et al., 2017b. Interaction of turmeric (*Curcuma longa* L.) with beneficial microbes: a review. *3 Biotech*, 7 (6), 357.
- Kumar, V., Kumar, A., Pandey, K.D., Roy, B.K., 2015b. Isolation and characterization of bacterial endophytes from the roots of *Cassia tora* L. *Ann Microbiol*, 65, 1391–1399.
- Li, X.Z., Nikaido, H., Williams, K.E., 1997. Silverresistant mutants of *Escherichia coli* display active efflux of Ag⁺ and are deficient in porins. *J. Bacteriol.* 179, 6127–6132.
- Lenka, M., Panda, K.K., Panda, B.B., 1992. Monitoring and assessment of mercury pollution in the vicinity of a chloralkali plant IV. Bioconcentration of mercury in in situ aquatic and terrestrial plants at Ganjam, India. *Arch Environ Contam Toxicol*, 22 (2), 195–202.
- López-Millán, A.F., Sagardoy, R., Solanas, M., Abadía, A., Abadía, J., 2009. Cadmium toxicity in tomato (*Lycopersicon esculentum*) plants grown in hydroponics. *Environ Exp Bot*, 65 (2), 376–385.
- Loutit, J.S., 1970. Investigation of the mating system of *Pseudomonas aeruginosa* strain I VI. Mercury resistance associated with the sex factor (FP). *Gen Res*, 16 (2), 179–184.
- Lutkenhaus, J.F., 1977. Role of a major outer membrane protein in *Escherichia coli*. *J Bacteriol.* 131, 631–637.
- Marschner, H., 1995. *Mineral Nutrition of Higher Plants*. Academic Press, London.

- Merdy, P., Gharbi, L.T., Lucas, Y., 2009. Pb, Cu and Cr interactions with soil: sorption experiments and modelling. *Colloids Surf A Physicochem Eng Asp*, 347 (1), 192–199.
- Miransari, M., 2011. Hyperaccumulators, arbuscular mycorrhizal fungi and stress of heavy metals. *Biotechnol Adv*, 29 (6), 645–653.
- Mishra, J., Singh, R., Arora, N.K., 2017. Alleviation of heavy metal stress in plants and remediation of soil by rhizosphere microorganisms. *Front Microbiol*, 8, 1706.
- Nies, D.H., 1999. Microbial heavy-metal resistance. *Appl Microbiol Biotechnol*, 51, 730–750.
- Oaikhena, E.E., Makaije, D.B., Denwe, S.D., Namadi, M.M., Haroun, A.A., 2016. Bioremediation potentials of heavy metal tolerant bacteria isolated from petroleum refinery effluent. *Am J Environ Prot*, 5 (2), 29–34.
- Opeolu, B.O., Adenuga, O.O., Ndakidemi, P.A., Olujimi, O.O., 2010. Assessment of phyto-toxicity potential of lead on tomato (*Lycopersicon esculentum* L) planted on contaminated soils. *Int J Phys Sci*, 5 (2), 68–73.
- Palacios, G., Gomez, I., Carbonell-Barrachina, A., Pedreño, J.N., Mataix, J., 1998. Effect of nickel concentration on tomato plant nutrition and dry matter yield. *J Plant Nutr* 21 (10), 2179–2191.
- Pathma, J., Kennedy, R.K., Sakthivel, N., 2011. Mechanisms of fluorescent pseudomonads that mediate biological control of phytopathogens and plant growth promotion of crop plants. In: Maheshwari, D. (Ed.), *Bacteria in Agrobiolgy: Plant Growth Responses*. Springer, Berlin, Heidelberg, pp. 77–105.
- Pugsley, A.P., Schnaitman, C.A., 1978. Outer membrane proteins of *Escherichia coli*. VII. Evidence that bacteriophage-directed protein 2 functions as a pore. *J. Bacteriol*. 133, 1181–1189.
- Rajkumar, M., Sandhya, S., Prasad, M.N.V., Freitas, H., 2012. Perspectives of plant-associated microbes in heavy metal phytoremediation. *Biotechnol Adv*, 30, 1562–1574.
- Rani, D.R., Mahadevan, A., 1994. Cloning and expression of the mercury resistance genes of marine *Pseudomonas* sp. strain MR1 plasmid pMR1 in *Escherichia coli*. *Res Microbiol*, 145 (2), 121–127.
- Richardson, A.E., Barea, J.M., McNeill, A.M., Prigent-Combaret, C., 2009. Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. *Plant Soil* 321 (1–2), 305–339.
- Sheldon, A.R., Menzies, N.W., 2005. The effect of copper toxicity on the growth and root morphology of Rhodes grass (*Chloris gayana* Knuth.) in resin buffered solution culture. *Plant Soil* 278 (1–2), 341–349.
- Turnau, K., Kottke, I., 2005. Fungal activity as determined by microscale methods with special emphasis on interactions with heavy metals. In: Dighton, J., White, J.F. (Eds.), *The Fungal Community*. CRC Press, Boca, Raton, pp. 287–305.
- Velásquez, L., Dussan, J., 2009. Biosorption and bioaccumulation of heavy metals on dead and living biomass of *Bacillus sphaericus*. *J Hazard Mater* 167 (1–3), 713–716.
- Vijayaraghavan, K., Palanivelu, K., Velan, M., 2006. Biosorption of copper (II) and cobalt (II) from aqueous solutions by crab shell particles. *Bioresour Technol*, 97, 1411–1419.
- Von Canstein, H., Li, Y., Timmis, K.N., Deckwer, W.D., Wagner-Döbler, I., 1999. Removal of mercury from chloralkali electrolysis wastewater by a mercury-resistant *Pseudomonas putida* strain. *Appl Environ Microbiol*, 65 (12), 5279–5284.
- Walsh, U.F., Morrissey, J.P., O’Gara, F., 2001. *Pseudomonas* for biocontrol of phytopathogens: from functional genomics to commercial exploitation. *Curr Opin Biotechnol*, 12 (3), 289–295.

- Wang, C.L., Michels, P.C., Dawson, S.C., Kitisakkul, S., Baross, J.A., Keasling, J.D., et al., 1997. Cadmium removal by a new strain of *Pseudomonas aeruginosa* in aerobic culture. *Appl Environ Microbiol*, 63 (10), 4075–4078.
- Wang, J., Chen, C., 2006. Biosorption of heavy metals by *Saccharomyces cerevisiae*: a review. *Biotechnol Adv*, 24, 427–451.
- Whipps, J.M., 2001. Microbial interactions and biocontrol in the rhizosphere. *J Exp Bot*, 52 (Suppl 1), 487–511.
- Wingender, J., Strathmann, M., Rode, A., Leis, A., Flemming, H.C., 2001. Isolation and biochemical characterization of extracellular polymeric substances from *Pseudomonas aeruginosa*. *Methods Enzymol* 336, 302–314.
- Winkelmann, G., Zahner, H., 1973. Stoffwechselprodukte von Mikroorganismen. 115. Mitteilung. Eisenaufnahme bei *Neurospora crassa*. I. Zur Spezifität des Eisentransportes. *Arch Microbiol*, 88, 4940.
- Zhang, Y.X., Wang, J., Chai, T.Y., Zhang, Q., Liu, J.G., Li, X., et al., 2012. Mechanism of heavy-metal tolerance in *Pseudomonas aeruginosa* ZGKD2. *Huan Jing Ke Xue*, 33 (10), 3613–3619.

FURTHER READING

- Gonzalez-Guerrero, M., Melville, L.H., Ferrol, N., Lott, J.N., Azcón-Aguilar, C., Peterson, R.L., 2008. Ultrastructural localization of heavy metals in the extraradical mycelium and spores of the arbuscular mycorrhizal fungus *Glomus intraradices*. *Can J Microbiol*, 54 (2), 103–110.
- Khan, S., Cao, Q., Zheng, Y.M., Huang, Y.Z., Zhu, Y.G., 2008. Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China. *Environ Pollut*, 152 (3), 686–692.

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PGPR Amelioration in Sustainable Agriculture

Food Security and Environmental Management

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PGPR Amelioration in Sustainable Agriculture: Food Security and Environmental Management explores the plant growth-promoting rhizobacteria (PGPR) that are indigenous to soil and plant rhizosphere. These microorganisms have significant potential as important tools for sustainable agriculture. PGPR enhance the growth of root systems as well as the entire plant and often control certain plant pathogens.

PGPR Amelioration for Sustainable Agriculture is a fascinating subject, multidisciplinary in nature, and focusing on plant health and plant protection. The book emphasizes the current trends of, and probable future, PGPR developments. The chapters incorporate both theoretical and practical aspects, and will serve as baseline information for future research.

This book will be useful to students, teachers, and researchers, both in universities and at research institutes, especially those working in areas of agricultural microbiology, plant pathology, and agronomy.

Key Features

- Presents new concepts and current developments in PGPR research and evaluates the implications for sustainable productivity.
- Describes the role of multiomics approaches in establishing understanding of plant–microbe interactions that help plants optimize abiotic stresses.
- Addresses intellectual property rights, and laws relating to biotechnological patents, copyrights, trademarks, trade secrets and other relevant concerns.



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